

**EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT
OF SOLANUM SEAFORTHIANUM LEAF EXTRACT
AGAINST CCl₄ - INDUCED LI-1**

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1. INTRODUCTION

MEDICINAL HERBS

Herbal medicine is the oldest form of health care system known to mankind and all living being including man has been troubling by many ailments from past so many centuries and most often nature has provided the cure. Herbs had been used by all cultures throughout history and are the important members of nature. Herbs has played the great role from years in combating the disease of human race.

Herbal medicine is a major component in all indigenous people's traditional medicine. In India the use of the medicinal herbs is as old as 1500BC. Medicinal herbs have been in use for thousands of years, in one form or other, under the indigenous system of medicine like Ayurveda, Unani and Sidha. India has 13,000-20,000 specious flowering plants, 2800 algae, 23,000 fungi, 1,600 types of latches, 1,800 varieties of bryophytes and an estimated 30 million types of microorganisms. There are few medicinal herbs of commercial importance, in context with the abundant flora in India. India is perhaps the large producer of medicinal herbs and is rightly called as "Botanical garden of the world". Herbal medicines are prepared from a variety of plant material such as leaves, stems, roots, barks, etc. They usually contain many biologically active ingredients and are considered to be medicinal if they possess pharmacological activities of possible therapeutic use. Today, herbal drugs are coming back into prominence and many drugs commonly used today care of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States of America contain at least

one active ingredient derived from plant materials. Side effects of the conventional medicines such as antibiotics, antimicrobial agents are the major problem. Over the years, some of the infectious organisms have developed resistance to synthetic drugs hence more and more complications. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural product having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor people.

Herbals are used in the art of healing since the time immemorial and the primitive man through trial and error gained knowledge of herbal and passed this information on to the next progeny. Despite the development in modern medicines, the use of herbs is still increasing. As the time went on, each tribe added the medicinal power of herbs in the area to its knowledge base. They methodically collected information on herbs that help in the development of well-defined herbal pharmacopoeias. Some of those in the current use have been an ancient heritage, whereas other has arisen from discoveries and cultural trends in more recent centuries.

According to the World Health Organization (WHO) more than 1 billion people rely to some extent on medicinal. The WHO has listed 22,000 plants around the world having medicinal values. The extensive advances and development of science of phyto-pharmaceuticals and hopes for remedies in chronic disease generated new enthusiasm in the research workers to develop herbal medicines. The major achievement in the field was the discovery of vincristine and vinblastine in the madagascars periwinkle and it

make marked difference in the treatment of cancer. Morphine, digitoxin, ergotamine and quinine are the some of the other excellent drugs that obtained from different plant species (Tyler et al.1998)

LIVER-INTRODUCTION

The liver has pivotal rule in human metabolism, including detoxification, protein synthesis, and the production of bio-chemicals necessary for digestion. The liver is necessary for survival; there is no currently way to compensate for the absence of liver function long term, however liver cell can regenerate.

It produces and secretes bile that stored in gallbladder which used to break down and digest fatty acids in food.

- It also produces prothrombin and fibrinogen, both are blood-clotting factors.
- It converts sugar into glycogen, which it stores until the muscle cells need energy and it is secreted into the blood stream as glucose.
- It synthesizes protein and cholesterol and convert carbohydrates and protein into fats, which is stored for later uses.
- It also produces blood protein and enzymes needed for digestion and other bodily functions in body.
- The liver stores critical trace elements such as iron, copper, as well as vitamins A, D, and B₁₂.
- The liver is responsible for detoxifying of poisonous substances in the body.

One of the primary functions of the liver is to aid in the metabolism of substance including food, dietary supplement, alcohol, and other medications. Due to its unique metabolism and closely related with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. 75% of blood coming to the liver arrives directly from the gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics in near-undiluted form and there several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damages mitochondria, an intracellular organelle that produce energy. Its dysfunction release excessive amount of oxidants which, in turn, injured the hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also lead to the oxidative stress. In fact, there are many types of liver disease that can be caused by virus, damage from drugs or chemicals, obesity, diabetes or an attack from your own immune system.

According to W.H.O about 25,000 people die in every year due to hepatic diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, hepatic encephalopathy, portal hypertension, fulminate hepatic failure and certain tumors like hepatoma in liver. It is estimated that 2 billions people around the world are infected with hepatitis B virus about 370 millions of these have the chronic form of the disease.

Medicinal herbs are significant source of pharmaceutical drug and the latest trends have shown increasing demand of phytodrugs and some medicinal herbs have been proven hepatoprotective potential. Silymarin, a flavonol lignin mixture extracted from *silybum marianum* (milk thistle) is a

popular remedy for hepatic diseases. The present study is an attempt to test the hepatoprotective activity of *Solanum seafortianum* leaf extract.

Anatomy of Liver.

The liver has a right lobe and a left lobe and inside the two lobes is a network of tubes, also called the biliary tree which carries bile from liver to intestine. The liver lies to the right of the stomach and overlies the gallbladder and it is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients materials from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries and which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells and that are the functional units of the liver.

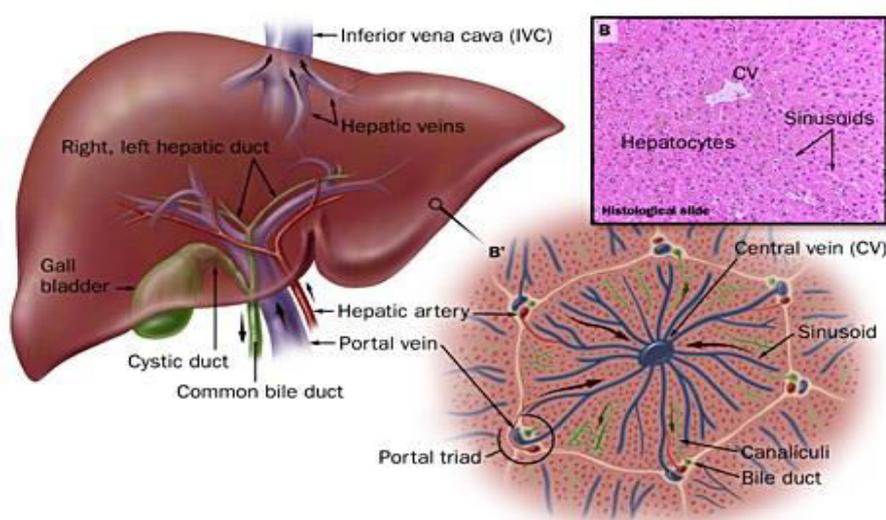


Figure 1. Anatomy of liver

Histology of liver

The basic functional unit of liver is the liver lobule, a cylindrical structure, several millimeters in length 0.8 mm. and 2 mm in diameter. The human liver contains 50,000 to 1, 00,000 lobules and which is constructed around a central vein which empties into a hepatic venule than the hepatic vein finally into inferior venacava. The hepatic lobule is roughly a hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center in liver.

The hepatic acinus which is oriented around the afferent vascular represents a unit that is consists of an irregular shaped. Roughly ellipsoidal mass of hepatocytes aligned around the hepatic arterioles and portal venules. The acinus is roughly divided into zones that correspond to distance from the arterial blood supply those hepatocytes closest to the arterioles are the best oxygenated, while those farthest from the arterioles have the poorest oxygen supply.

Hepatocytes are the major functional cells of the liver and are polygonal in shape. Hepatic cells sides can be in contact either with sinusoids (sinusoidal face) or neighboring hepatocytes (lateral faces) the mid perform a number of metabolic endocrine and secretory functions. Roughly 80% of the mass of the liver is contributed by hepatocytes and are exceptionally active in synthesis of protein and lipids for export and also synthesize and secrete very low density lipoproteins.

Another type of particle observed in copious quantities in liver is glycogen. It is a polymer of glucose and the density of its aggregates in

hepatocytes varies dramatically depend on whether the liver is examined shortly after the meal (abundant glycogen) or following a prolonged fast (minimal quantities of glycogen).

Sinusoids are the canals formed by the plates of hepatocytes. They are approximately 8-10 μ m in diameter and comparable with the diameter of normal capillaries. They are orientated in a radial direction in the lobule. Sinusoids are low-pressure vascular channels that receive blood from terminal branches of the hepatic artery and portal vein at the periphery of lobules and deliver it into central veins. Sinusoids are lined with endothelial cells and flanked by plates of hepatocytes. The space between sinusoidal endothelium and hepatocytes is called the space of Disse. Sinusoidal endothelial cells are highly fenestrated, which allows virtually unimpeded flow of plasma from sinusoidal blood into the space of Disse.

Functions of the liver

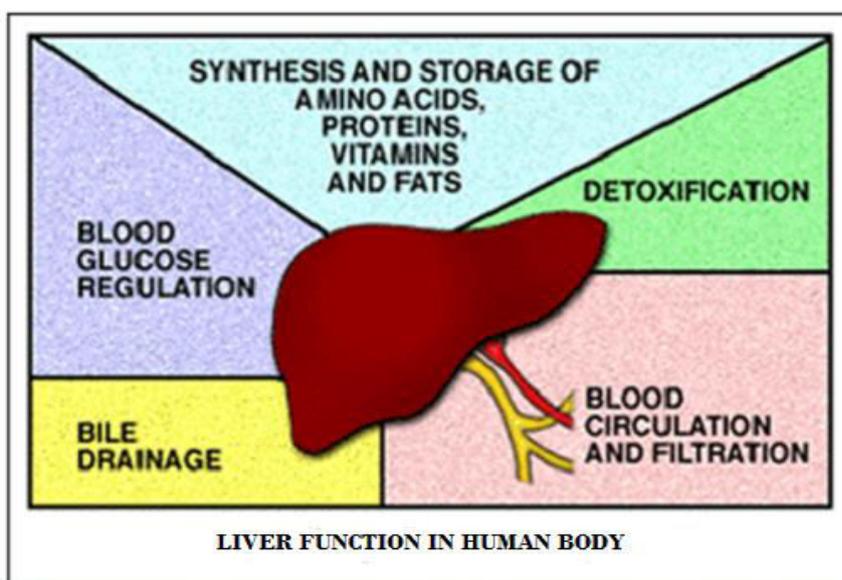


Figure 2. Functions of the liver

❖ Carbohydrate metabolism

- Gluconeogenesis
- Glycogen synthesis & metabolism

The liver is especially important in maintaining a normal blood glucose level in body and can break down glycogen to glucose and release glucose into the bloodstream when blood have low glucose level. The liver also can convert amino acids into lactic acid and galactose into glucose. The liver converts glucose to glycogen and triglycerides for storage when blood glucose is high, as occurs just after having a meal.

❖ Lipid metabolism

- Fatty acid synthesis
- Cholesterol synthesis & excretion
- Lipoprotein synthesis.
- Ketogenesis.(converting) The fatty acid ketone bodies.

Hepatocytes store some triglycerides, break down fatty acids to generate ATP, synthesize lipoproteins, transport fatty acids, triglycerides and cholesterol to and from the body cells, synthesize and use cholesterol to make bile salts.

❖ Protein metabolism

- Synthesis of plasma proteins.
- Urea synthesis.

Hepatocytes de-amine (remove the amino group, NH₂) amino acids so that amino acid can be used for the production ATP or converted to

carbohydrate or fats. The resulting toxic ammonia is which is then converted into the much less toxic urea and excreted in urine. Hepatocytes can also synthesize most plasma proteins, such as alpha and beta globulins, albumin, prothrombin and fibrinogen.

❖ **Processing of drugs and hormones:**

- Metabolism , conjugation & excretion of steroidal & poly peptide hormones

The liver can detoxify substances such as alcohol or excrete drugs like penicillin, erythromycin, and sulfonamides into the bile. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogen and aldosterone.

❖ **Excretion of Bilirubin:**

Bilirubin, derived from the blood cells is absorbed by the liver from the blood stream and secreted into bile. Most of the bilirubin in bile is metabolized in the small intestine by bacteria and eliminated in to feces.

❖ **Phagocytosis:**

The stellate reticulo-endothelial (Kupffer's) cells of the liver phagocytose aged white blood cells, red blood cells and some bacteria.

❖ **Storage**

Liver is a good part in storage of glycogen, vitamin A, vitamin B₁₂ and iron

LIVER DISEASES

Liver diseases are considered as fatal & life threatening and it creates a serious challenge to public health. Liver diseases are due to infection and /

or exposure of liver to various toxic substances such as different drugs or alcohol. Some times over dosage of some drugs can also lead to liver damage. Now-a-days due to inadequacy of liver protective agents, researchers and traditional medicine practitioners are concentrate in herbal based remedies for the various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders so there was no safe hepatoprotective drug available for the treatment of liver disorders. Therefore, many folk remedies from plantsource are used for the protection of hepatic disease starting from ancient period. Liver diseases remain as one of the serious health problems however we do not have satisfactory liver protective drugs in allopathic medical practice so herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as traditional system of medicine in India.

Liver disease which cause an acute or chronic damage to the liver, usually caused by infection, injury, exposure to many drugs or toxic compounds, an autoimmune process, or by a genetic defect such as haemochromatosis. The disease can also be categorized according to the effect it has on the liver. Hepatitis is an inflammation of the liver, cirrhosis involves scarring and progressive hepatic cell death, stones can be developed and which form blockages, fatty liver and cancer, which can be life threatening. Genetic defect cause liver disease by preventing vital functions or by depositing substances the liver cell.

➤ **HEPATITIS:**

There are two major forms of hepatitis

One in which the liver is damaged quickly (called as acute hepatitis) and the other in which the liver is damaged slowly only, over a long time (called as chronic hepatitis).

While hepatitis can be caused by any of the means mentioned above, most commonly it is due to infection by several viruses that damage the liver, termed hepatitis viruses. These Viruses have been named in the according to their discovery as hepatitis A, B, C, D, and E.

➤ **Hepatitis A**

This infection is usually caused by consuming contaminated water or food and is especially common in children. Most infected people do not even know about they have been exposed to the virus.

➤ **Hepatitis B**

It is fairly common disease and still the most common cause of acute viral hepatitis. Hepatitis B can spread one person to other by exposure to blood, through sexual relations, and from mother to baby. In a small number of cases, Hepatitis B can develop into a chronic infection and vaccine to prevent infection is available.

➤ **Hepatitis C**

It is less common than hepatitis B as a cause of acute hepatitis, but in some cases the majority of the people who contract it become chronically infected, hepatitis c can able to spread through contact with infected blood, Hepatitis C infection can be very serious. Most people infected experience

no symptoms but the virus may remain in the liver for years and it is not discovered until much damage is done. Up to fifty percent of those infected with Hepatitis C are able to fight off the virus within six months. However, many patients become develop a chronic infection.

Hepatitis D and E are rare.

➤ **CIRRHOSIS**

Anything that causes severe ongoing injury to the hepatic cells can lead to cirrhosis. It is marked by cell death or scar formation and which lead to a progressive disease that creates irreversible damage of liver. Cirrhosis is treated by trying to limit further damage to liver and the main causes of cirrhosis include chronic excessive alcohol intake; auto immune diseases, it can be cause fluid build-up in the abdomen called as cites, muscle wasting, bleeding from the intestines easy bruising, enlargement of the breasts in men called as gynecomastia, and a number of other problems may be occur.

➤ **GALLSTONES**

Cholesterol and bile pigments (bilirubin) in the bile may form stone in the gallbladder. If present for a long time, they may damage the gall bladder and may block the duct that origins bile from the gallbladder which causing sharp pain to develop suddenly in the upper right pan of the abdomen, and in some cases, leading of the gallbladder and fever.

➤ **OBSTRUCTION**

Gallstones, trauma, tumors, and inflammation can cause blockage or obstructions in the ducts draining the liver .When an obstruction occurs, bile

and its associated wastes accumulate in the liver cells and the patient's skin and eyes often turn to yellow (Jaundice) bilirubin in the urine turns to dark brown in color.

➤ **FATTY LIVER**

Fatty liver which causes liver enlargement, tenderness, and abnormal liver functions and the common cause is excessive alcohol consumption. It is usually a reversible condition, resolving with abstention from alcohol. Another cause of fatty liver is NASH (Non Alcoholic Steato Hepatitis) the most common chronic hepatitis is not caused by viruses. It is seen commonly in overweight and diabetic individuals. Excess liver fat can lead to inflammation, progressing to scarring (or cirrhosis) in 25 percent of patients. Treatment includes weight loss, exercise, regulating blood pressure, diabetes control and cholesterol, limiting alcohol consumption and not smoking.

➤ **LIVER CANCER**

Hepatitis and cirrhosis may leads to liver cancer in some cases only, but cancer from other parts of the body that spreads to the liver is more common cause of liver cancer. It is better people who have chronic hepatitis or cirrhosis may be checked on a regular basis for cancer, often with an Alpha-Fetoproteins (AFP) test or an ultrasound. Infiltrative conditions which can be affect the liver include lymphomas and amyloidosis.

➤ **INHERITED ABNORMALITIES IN METABOLISM**

There are four conditions in which jaundice is caused by inherited abnormality of Bilirubin metabolism are gilberts, crigler – najjar , Dubin –

Johnson & rotor syndromes. Gilbert's syndrome affects 2-3% of the population but the others are rare and the patient with Gilbert's syndrome has decreased conjugation of bilirubin. The jaundice of Gilbert's syndrome is typically mild & present only intermittently. It is often noticed after an infection or a period of decreased food intake. The liver will be histologically normal.

Wilson's disease is an inherited abnormality of copper metabolism in the liver and characterized by decreased biliary excretion of copper. Copper is deposited in the liver and patients with Wilson's disease may present either in childhood with hepatitis accompanied by haemolysis & renal tubular defect or in young adults with cirrhosis may be seen.

Hereditary Hemochromatosis – The common adult genetic liver disease in which a specific genetic defect leads to iron accumulation in the liver cells, leading to liver cancer and liver cirrhosis in some patients. Iron accumulation may go beyond the liver affecting the joints, heart and pancreas. Specific effective treatments are available for Hemochromatosis. Liver transplantation may be required in some of these patients.

Alpha-1 antitrypsin deficiency (Alpha-1) – This inherited disease affects the liver and the lungs in patients. It is caused by an inability to produce enough amount of a particular protein, called Alpha-1 antitrypsin, which is used to prevent the breakdown of some enzymes in various

organs. Management of Alpha-1 antitrypsin deficiency includes maintaining normal nutrition, patient education.

HEPATOTOXICITY

Toxic liver injury produced by some drugs and chemicals may virtually mimic any form of naturally occurring liver disease. Severity of hepatotoxicity is greatly increased if the drug is continued after symptoms develop.

Arsenic, copper, phosphorus and iron are among the various inorganic compounds producing hepatotoxicity. The organic agents include certain naturally- occurring plant toxins such as pyrrolizidine, mycotoxins, alkaloids and bacterial toxins. In addition, exposure to hepatotoxin compounds may environmental, occupational or domestic that could be accidental, homicidal or suicidal ingestion.

MECHANISM OF HEPATOTOXICITY CAUSED BY DIFFERENT AGENTS

Damage to the liver is not due to the drug itself but due to a toxic metabolite N-acetyl-p benzoquinonimine (NABQI/NAPQI) which is produced by cytochrome P 450 enzymes in liver.

In overdoses large amount of NAPQI is generated which overwhelm the detoxification process and lead to damage to hepatic cells. Nitric acid also plays some role in inducing toxicity. Hepatotoxicity caused by NSAIDs were documented to be both idiosyncratic and dose dependant eg; Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity and idiosyncratic reaction has been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin. Enlarged liver cells is a rare side effect of long term use of steroid in children.

Carbon tetrachloride

Liver injury due to carbon tetrachloride in rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P 450 in endoplasmic reticulum and mitochondria with the formation of CCl_3O^- , a reactive oxidative free radical, which initiates lipid peroxidation.



It is postulated that secondary mechanisms link carbon tetrachloride metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from carbon tetrachloride metabolism or from peroxidative degeneration of membrane lipids. The possible involvement of radical species such as trichloromethyl ($\cdot CCl_3$), trichloromethylperoxy ($\cdot OOCCL_3$), and chlorine ($\cdot Cl$) free radicals, as well as phosgene and aldehydic products of lipid peroxidation, as toxic intermediates

Administration of a single dose of CCl_4 to a rat produces, within 24 hrs, a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thereafter, the level falls and by 24 hrs there is no CCl_4 left in the liver. The development of necrosis is associated with leakage of hepatic enzymes into serum.

Dose of CCl_4 : 0.1 to 3 ml/kg I.P.

Galactosamine

Galactosamine produces diffuse type of liver injury simulating viral hepatitis. It presumably disrupts the synthesis of essential uridylyate nucleotides resulting in organelle injury and ultimately cell death. Depletion of those nucleotides would impede the normal synthesis of RNA and consequently would produce a decline in protein synthesis. The cholestasis caused by galactosamine may be from its damaging effects on bile ducts or ductless or canalicular membrane of hepatocytes. Galactosamine decrease the bile flow and its content i.e. bile salts, cholic acid and deoxycholic acid. Galactosamine reduces the number of viable hepatocytes as well as rate of oxygen consumption.

Dose of D-Galactosamine: 400 mg/kg, P.

Thioacetamide

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. A metabolite of thioacetamide (perhaps s-oxide) is responsible for hepatic injury. Thioacetamide reduce the number of viable hepatocytes as well as rate of oxygen consumption. It also decreases the volume of bile and its content i.e. bile salts, cholic acid and deoxycholic acid.

Dose of Thioacetamide: 100 mg/kg, S.C.

Alcohol

The effects of ethanol have been suggested to be a result of the enhanced generation of oxyfree radicals during its oxidation in liver. The

peroxidation of membrane lipids results in loss of membrane structure and integrity. This results in elevated levels of i-glutamyltranspeptidase, a membrane bound enzyme in serum. Ethanol inhibits glutathione peroxidase, decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver. The decrease in activity of antioxidant enzymes superoxide dismutase, glutathione peroxidase are speculated to be due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, formed by oxidation of ethanol. Alcohol pre-treatment stimulates the toxicity of CCl₄ due to increased production of toxic reactive metabolites of CCl₄, namely trichloro-methyl radical by the microsomal mixed function oxidative system. This lipid peroxidative degradation of bio membranes is the principle cause of hepatotoxicity.

Paracetamol

Paracetamol, a widely used analgesic and antipyretic drug, Damage to the liver is not due to the drug itself but to a toxic metabolite (N-acetyl-p-benzoquinone imine NAPQI, or NABQI) which is produced by cytochrome P-450 enzymes in the liver. In normal circumstances, this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In an overdose, a large amount of NAPQI is generated which overwhelms the detoxification process and leads to necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Although individual analgesics rarely induce liver damage due to their widespread use, NSAIDs have emerged as a major group of drugs exhibiting hepatotoxicity. Both dose-dependent and idiosyncratic reactions have been documented. Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity; idiosyncratic reaction has been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin.

Glucocorticoids

Glucocorticoids are so named due to their effect on the carbohydrate mechanism. They promote glycogen storage in the liver. An enlarged liver is a rare side effect of long-term steroid use in children. The classical effect of prolonged use both in adult and paediatric population is steatosis.

Isoniazid

Isoniazide (INH) is one of the most commonly used drugs for tuberculosis; it is associated with mild elevation of liver enzymes in up to 20% of patients and severe hepatotoxicity in 1-2% of patients.

Natural products

Examples include many amanita mushrooms (particularly the destroying angels), and aflatoxins

SCREENING MODELS

In vivo models.

A toxic or repeated doses of a known hepatotoxin (carbon tetrachloride, paracetamol, thioacetamide, alcohol, D-galactosamine etc.) might be administered, to induce liver damage in experimental animals. The test substance (plant extract) is administered along with, prior to and/or after the toxin treatment. Assessment of liver damage and recovery from damage are by quantifying serum marker enzymes, bilirubin, bile flow, histopathological changes and biochemical changes in liver. An augmented level of liver marker enzymes such as serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase in the serum indicates liver damage. Additionally, hepatotoxicity may result in decline of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromsulphthalein may be used in the assessment of hepatoprotective action of plants. Efficacy in therapy of a drug against diverse hepatotoxins differs especially when their mechanism of action vary. Consequently, the efficacy of each drug has to be tested against hepatotoxins which act by varied methods. Hepatotoxic chemicals like carbon tetrachloride, paracetamol reduces the level of metabolizing enzymes in liver. For the above reason, metabolism of hexobarbitone is reduced resulting in prolongation of hexobarbitone induced sleeping time. Hence, reduction in carbon tetrachloride - induced prolongation of hexobarbitone stimulated sleeping time may be exploited to screen anti-carbon tetrachloride toxicity of drugs in animals. Care has to be taken to see that the drug has no direct effect on drug metabolizing enzymes or necrosis.

In vitro studies

Fresh hepatocyte preparations and primary cultured hepatocytes may be exploited to study the anti-hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An augmented activity of marker transaminases in the medium indicates liver damage. Parameters such as hepatocytes multiplication, morphology, macromolecular synthesis and oxygen consumption are determined. Effective antiviral assays using cell culture and Polymerase Chain Reaction techniques remain to be explored and these may emerge as a very promising strategy for in vitro examination of hepatoprotective effect of herbal products. **(Varshakashaw et al., 2011)**

LIVER FUNCTION TESTS

As the liver performs its various functions and it makes chemicals that pass into the bloodstream and bile. Some of these chemicals can be measured in a blood sample and tests that are commonly done on a blood sample are called liver function tests (LFTs). These usually measure the following:

The various uses of Liver function tests include:

Screening: liver function test are a non-invasive yet sensitivescreening modality for liver dysfunctions.

Pattern of disease: liver function test are helpful to recognize the pattern of liver disease also helpful in differentiating between various disease such as acute viral hepatitis, cholestatic disorders and chronic liver disease. (CLD).

Assess severity: liver function test are helpful to assess the severity and predict the outcome of certain diseases like primary biliary cirrhosis.

Follow up: liver function test are helpful in the follow up of certain liver diseases and also helpful in evaluating response to therapy like autoimmune hepatitis.

CLASSIFICATION OF LIVER FUNCTION TESTS

A. Tests of the liver's capacity to transport organicanions and to metabolize drugs-

Serum bilirubin, urinebilirubin, urobilinogen *etc...*

B. Tests that detect injury to hepatocytes (serum enzymetests) –
Aminotransferases, alkaline phosphatase, ãglutamyl transpeptidase, 5-nucleotidase, leucineaminopeptidase *etc....*

C. Tests of the Liver's biosynthetic capacity-

Serumproteins, albumin, prealbumin, serum ceruloplasmin,procollagen III peptide, antitrypsin, feto protein,prothrombin time *etc...*

SERUM BILIRUBIN

Bilirubin is an endogenous anion which derived from haemoglobin degradation from the RBC. Measuring bilirubin by direct and indirect bilirubin are based on the original van der Bergh method. Bilirubin is altered when exposure to light so serum and plasma samples must be kept in dark before measurements are made. When the liver function tests are abnormal and the serum bilirubin levels more than $17\mu\text{mol/L}$ suggest underlying some liver disease. (Friedman SF et.al. 2003)

Types of bilirubin

i. Total bilirubin: This is measured as the amount, which reacts in 30 minutes after addition of alcohol. Normal range is 0.2-0.9 mg/dl ($2-15\mu\text{mol/L}$) and it is slightly higher by 3-4 $\mu\text{mol/L}$ in males as compared to females. Which helps to diagnose Gilbert syndrome in males easily.

ii. Direct Bilirubin: This is the water-soluble fraction. Direct bilirubin is measured by the reaction with diazotized sulfanilic acid in 1 minute and it gives estimation of conjugated bilirubin. Normal range 0.3mg/dl ($5.1\mu\text{mol/L}$)

iii. Indirect bilirubin: This fraction is calculated by the difference of the total and direct bilirubin and is a measure of unconjugated fraction of bilirubin. (Daniel SP, Marshall MK. 1999)

The elevated level of bilirubin which shows the decrease the hepatic clearance, the mild elevation due to liver disease physiological jaundice, inherited hyperbilirubinemias and moderate elevation due to drugs, viral hepatitis, inherited hyperbilirubinemias, Indiazo method of bilirubin estimation

is not very accurate especially in detecting low levels of bilirubin. Direct bilirubin over estimates bilirubin esters at low bilirubin levels and under estimates them at high concentration. Thus slight elevation of unconjugated bilirubin not detected, which is of value in detecting conditions like Gilbert syndrome. (Rosalki SB et.al 1999)

A. ENZYMES THAT DETECT HEPATOCELLULAR NECROSIS – AMINOTRANSFERASES

The aminotransferases (formerly transaminases) are specific indicators of hepatocellular necrosis. These enzymes- aspartate amino transferase (AST, formerly serum glutamateoxaloacetic transaminase-SGOT) and alanine aminotransferase (ALT, formerly serum glutamic pyruvate transaminase-SGPT) catalyze the transfer of the amino acids of aspartate and alanine respectively to the α ketogroup of ketoglutaric acid. ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, kidney, skeletal muscle, brain and liver. (Rosen HR, Keefe EB 2000).

Alanine transaminase (ALT): This is an enzyme that helps to process proteins. (An enzyme is a protein that helps to speed up chemical reactions) Large amounts of ALT occur in liver cells but when the liver is injured or inflamed (as in hepatitis), the blood level of ALT usually rises and elevated level in ALT which shows leakage from damaged tissue

Aspartate aminotransferase (AST): This is another enzyme usually found inside liver cells. When a blood test detects high levels of this enzyme in the

blood it usually means the liver is injured in some way. However AST can also be released if heart or skeletal muscle is damaged

Alkaline phosphatase (ALP):.

Average values of alkaline phosphatase may vary with age and are relatively high in childhood and puberty and lower in middle age and higher again in old age. Males usually have higher values when compared to females. The levels of the enzyme correlate with person's weight and inversely with the height of the person, (Gordon T 1993). The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions, (Kaplan MM 1986), and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in bone, intestine and liver. In acute viral hepatitis level of alkaline phosphatase is usually either normal or moderately increased. Hepatitis A may present a cholestatic picture with marked and prolonged itching and elevation of alkaline phosphatase. Tumours may secrete alkaline phosphatase into plasma and there are tumour specific isoenzymes such as Regan, Nagao and Kasahara isoenzymes. Elevated serum levels of intestinal alkaline phosphatase have been found in patients with cirrhosis, particularly those with blood group type O, and may be associated specifically with intra hepatic disease as opposed to extrahepatic obstruction. (Warnes TW 1977).

Hepatic and bony metastasis can also cause elevated levels of alkaline phosphatase. Other diseases like infiltrative liver diseases, granulomatous liver disease, abscesses, and amyloidosis may also cause a

rise in alkaline phosphatase. Mildly elevated levels of alkaline phosphatase may be seen in cirrhosis and hepatitis of congestive cardiac failure. Low levels of alkaline phosphatase occur in hypothyroidism, pernicious anemia, zinc deficiency and congenital hypophosphatasia.

Albumin: This is the main protein made by the liver, and it circulates in the bloodstream. The ability to make albumin (and other proteins) is affected in some types of liver disorder. Corticosteroids and thyroid hormone stimulates albumin synthesis. A low level of blood albumin occurs in some liver disorders like cirrhosis, ascites.

Total protein: This measures albumin and all other proteins in blood including alpha 1 antitrypsin, alpha feto protein,

Bilirubin: This chemical gives bile its yellow/green colour. A high level of bilirubin in blood will make jaundiced ('yellow'). Bilirubin is made from haemoglobin. Haemoglobin is a chemical in red blood cells that is released when the red blood cells break down. Liver cells take in bilirubin and attach sugar molecules to it. This is then called 'conjugated' bilirubin which is passed into the bile ducts.

A raised blood level of 'conjugated' bilirubin occurs in various liver and bile duct conditions. It is particularly high if the flow of bile is blocked. For example, by a gallstone stuck in the common bile duct, or by a tumour in the pancreas. It can also be raised with hepatitis, liver injury, or long-term alcohol abuse.

A raised level of 'unconjugated' bilirubin occurs when there is excessive breakdown of red blood cells. For example, in haemolytic anaemia.

Role of Liver function tests

- To diagnose liver disorders.
- To monitor the activity and severity of liver disorders.
- As a routine precaution after starting certain medicines to check that they are not causing liver damage as a side-effect.

SYNTHETIC HEPATOPROTECTIVE DRUGS

There are no specific allopathic medicines used as hepatoprotective, although different research works are going on. Some drug like that Rimonabant chemically described as N-peperidino- 5 (4-chlorophenyl)-l-(2,4-dichlorophenyl)-4-ethylpyrazole-3-carboxamide, isselective endocannabinoid (CBI) receptor antagonist, inhibits the pharmacological effects of cannabinoids agonists in vitro and vivo and has hepatoprotective activity against hepatotoxicant like ethanol. It has seen that administration of rimonabant at 2.5mg/kg, 5mg/kg and 10mg/kg dose level attenuated the increased level of the serum enzymes, produced by Ethanol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment. Steroids like corticosteroids are under the study for their hepatoprotective action. Many other therapeutic interventions have been studied in alcoholic hepatitis, but have not been able to show convincing benefit, including trials of anti-oxidants (vitamin E, and combination anti-oxidants), anti-fibrotics (colchicine), anti-thyroid drugs, promoters of hepatic regeneration (insulin and glucagon), anabolic steroids (oxandrolone and testosterone), as well as calcium channel blockers (amlodipine), polyunsaturated lecithin, and a number of complementary and alternative medicines. A number of other agents have been tested in patients including

propylthiouracil to decrease the hyper-metabolic state induced by alcohol. Herbal drugs are more widely used than allopathic drugs as hepatoprotectives because they are inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects. In other hand side effects, interactions and toxicity of synthetic medicines vary wildly from mild to severe include insomnia, vomiting, fatigue, dry mouth, diarrhea, constipation, dizziness, suicidal thoughts, hostility, difficulty sitting still, depression, mania, seizures, coma, anemia, hair loss, high blood sugar, shoplifting, swelling, impotency, panic attacks, confusion

Herbal approach to treat Hepatic diseases:

In case of hepatic disease treatment herbal drugs are widely used than synthetic drugs. There are various herbal plants reported as hepatoprotective which are as follows.

Taraxacum officinale

Traditionally *Taraxacum officinale* has been used as a remedy for jaundice and other disorders of the liver and gallbladder, and as a remedy for counteracting water retention. Generally, the roots of the plant have the most activity regarding the liver and gallbladder. Oral administration of extracts from the roots of *Taraxacum officinale* has been shown to act as a cholagogue, increasing the flow of bile. Bitter constituents like taraxecerin and taraxcin are active constituents of the medicinal herb.

Cichorium intybus

Cichorium intybus is a popular Ayurvedic remedy for the treatment of liver diseases. It is commonly known as kasni and is part of polyherbal formulations used in the treatment of liver diseases. In mice, liver protection was observed at various doses of *Cichorium intybus* but optimum protection was seen with a dose of 75 mg/kg given 30 minutes after CCl₄ intoxication. In preclinical studies an alcoholic extract of the *Cichorium intybus* was found, to be effective against chlorpromazine-induced hepatic damage in adult albino rats. A bitter glucoside, Cichorin (C₃₂H₄₀O₁₉) has been reported to be the active constituent of the herb.

Solanum seafortianum

Aromatic water extracted from the drug is widely prescribed by herbal vendors for liver disorders. Although clinical documentation is scarce as far as hepatoprotective activity is concerned, but some traditional practitioners have reported favorable results with powdered extract of the plant.

Glycyrrhiza glabra

Glycyrrhiza glabra, commonly known as licorice contains triterpene saponin, known as glycyrrhizin, which has potential hepatoprotective activity. It belongs to a group of compounds known as sulfated polysaccharides. Several studies carried out by Japanese researchers have shown glycyrrhizin to be for anti-viral and it has potential for therapeutic use in liver disease.

Wilkstroemia indica

W. indica is a Chinese herb and has been evaluated in patients suffering from hepatitis B. A dicoumarin, daphnoretin is the active constituent of the herb. The drug has shown to suppress Hbs AG in Hep3B cells. It is said to be an activator of protein kinase.

Curcuma longa

Like Silymarin, turmeric has been found to protect animal livers from a variety of hepatotoxic substances, including carbon tetrachloride, galactosamine, pentobarbital, 1-chloro-2, 4-dinitrobenzene, 4-hydroxy-nonenal, and paracetamol. Diarylhepatonoids including Curcumin is the active constituent of the plant.

Tephrosia purpurea

In Ayurveda, the plant is known as *sharpunkha*. Alkali preparation of the drug is commonly used in treatment of liver and spleen diseases. In animal models, it offered protective action against carbon tetrachloride and D-galactosamine poisoning. The roots, leaves and seeds contain tephrosin, deguelin and quercetin. The hepatoprotective constituent of the drug is still to be proved.

foeniculum vulgare

Fennel (*Foeniculum vulgare* Mill. family Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, the leaves, stalks and seeds (fruits) of the plant are edible. Hepatoprotective activity of *Foeniculum vulgare* essential oil was studied using a carbon tetrachloride-induced liver fibrosis model in rats. The hepatotoxicity produced by chronic carbon

tetrachloride administration was found to be inhibited by *Foeniculum vulgare* essential oil with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin.

1.10 FREE RADICALS

Types of free radicals

Free radicals can be defined as molecules containing a single unpaired electron in atomic or molecular orbits. These molecules are highly reactive ion and have an important role in the pathogenesis of tissue damage in various disorders such as hepatic dysfunction, mastitis, kidney damage, inflammation, immune injury and carcinogenesis (Abd Ellah, 2010). The most important free radicals include superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$), and hypochlorous acid (HOCL) (Stohs, 1995). HOCL is produced by the reaction of hydrogenperoxide (H_2O_2) with chloride ions and plays an important role in the leukocyte respiratory burst, which is involved in the host defense system (Lunec, 1990). Nitric oxide ($NO\cdot$) acts as a free radical and as a biological mediator in biochemical reactions. Physiologically it is synthesized from L-arginine by NO synthase employing cofactor NADPH. In the host, NO arises in some pathological situations, such as sepsis, stroke, myocardial depression, and inflammatory responses, (Bredt & Snyder, 1994).

Superoxide anion induces important reducing reactions in biological materials via Fenton like reactions, which are catalyzed by redox cycling

metal ions, including iron, copper, chromium and vanadium, Stohs & Bagchi(1995). These metal ions have the ability to accept and donate single electrons, making them important catalysts of free radical reactions, the most widely distributed and most commonly studied transition metal ions are the cations iron and copper, (Stohs,1995). Superoxide anion reduces Fe^{3+} in metalloproteins such as ferritin. The reduction of protein bound iron is an important reaction in biological material, because if there is sufficient H_2O_2 available, a reaction between the resultant Fe^{2+} and H_2O_2 occurs and gives rise to the highly reactive $\cdot OH$ Lunec (1990). H_2O_2 traverses biological membranes and intracellularly targets phospholipids, carbohydrates, metalloproteins and DNA, and causes damage via Fenton's reaction, (Samuni et al,1981). The main characteristics are short living, unstable, react with other molecules to achieve stability.

Sources of free radicals

Free radicals may be released due to the by-product of cellular respiration, synthesized by enzyme system (phagocytic cell, neutrophils and macrophages), exposure to ionizing radiation, smoking, herbicides, pesticides, fried foods or the production by chain reaction and normal cellular respiration,

Free radicals may be produced in the liver as a subsequence to hepatic detoxification of drugs, chemicals and toxic materials, (Feher et al.1992) (Ogino & Okada,1995). The formation of oxygen free radicals may be physiological as in phagocytosis (superoxide and H_2O_2 are used by phagocytic cells to kill bacteria), a side effect of metabolic pathways, or may

occur in pathological conditions due to toxic agents as in the case of ischemia, inflammation, disease, or due to decreased antioxidant defences. (Miller et al,1993).

Mitochondria considered a major source for the production of O_2^- and H_2O_2 , about 2-3% of consumed oxygen is constantly converted into reactive oxygen/reactive nitrogen species(ROS/RNS) in the mitochondria, hepatocytes contain many mitochondria and therefore, generate excess ROS/RNS. (Stohs,1995).

In many liver diseases, including the wide range of neonatal hepatitis, the tissue inflammatory infiltrates are likely to be responsible for the formation of O_2^- , H_2O_2 , $\cdot OH$, $HOCl$ and the highly cytotoxic monochloramine. (Southorn & Powis,1988)and (McCord,1993).

In turn, the superoxide anion attracts further neutrophils to the inflammatory site by a chemotactic activity, causing an increase in tissue injury (Petroni et al., 1980). In addition ,activated macrophages, Kupffer cells and vascular endothelium can generate nitric oxide,which may react with superoxide generating peroxynitrite. The latter is responsible for the inhibition of mitochondrial respiration and DNA synthesis. (Moncada & Higgs,1993).

Liver damage due to iron (hemochromatosis) and copper overload is believed, at least partially, to derive from the catalytic activity of these metals in the Fenton reaction leading to the generation of ROS and increased lipid peroxidation with consequent abnormal mitochondrial function (Bacon et al., 1993; Sokol et al., 1993; Sokol et al., 1994).

Excessive free radical production has been involved in the occurrence in several disease processes.

1. drug toxicities
2. inflammation
3. aging
4. fibrosis
5. carcinogenesis
6. lipid peroxidation cellular membranes
7. implicated in several specific diseases
 - a. atherosclerosis
 - b. degenerative neurologic disease
 - c. reperfusion injury
8. oxygen toxicity

Sources of ROS in the liver are summarized in Fig. 3.

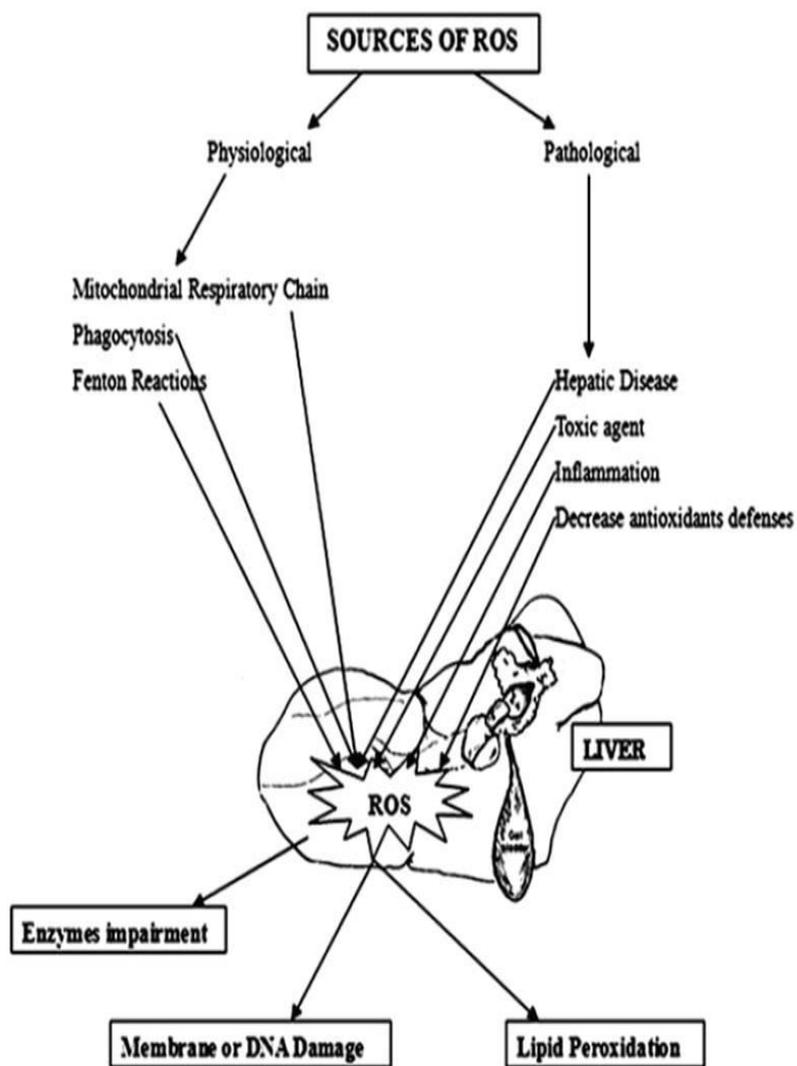


Figure. 3. Sources of reactive oxygen species (ROS) in the liver

(Abd Ellah et al., 2007)

Antioxidants

The cells contain a variety of antioxidant mechanisms that play a central role in the protection against reactive oxygen species. (Pár & Jávör,1984) and (Halliwell et.al 1991). The antioxidant system consists of antioxidant enzymes (superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase (GSH-Px)), glutathione, ancillary enzymes (glutathione reductase (GR), glutathione S-transferase, and glucose 6-phosphate dehydrogenase (G6PD)),metal-binding proteins (transferrin, ceruloplasmin and albumin), vitamins (α -tocopherol,ascorbate and beta-carotene), flavonoids, and urate (Halliwell, 1994).Pathological free radical reactions do not necessarily cause cell and tissue damage, asantioxidants of cells and tissues are able to prevent free radical injury. (Feher et al,1992). Onthe intracellular level, ROS formation and metabolism can be summarized as shown in Fig 2.

Hepatic oxidative stress and lipid peroxidation

The term “oxidative stress” has been coined to represent a shift towards the pro-oxidants in the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins,radiation, or strenuous physical activity. Protection against all of these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet.

Consequently, an inadequate intake of antioxidant nutrients may compromise antioxidant potential, thus compounding overall oxidative stress.

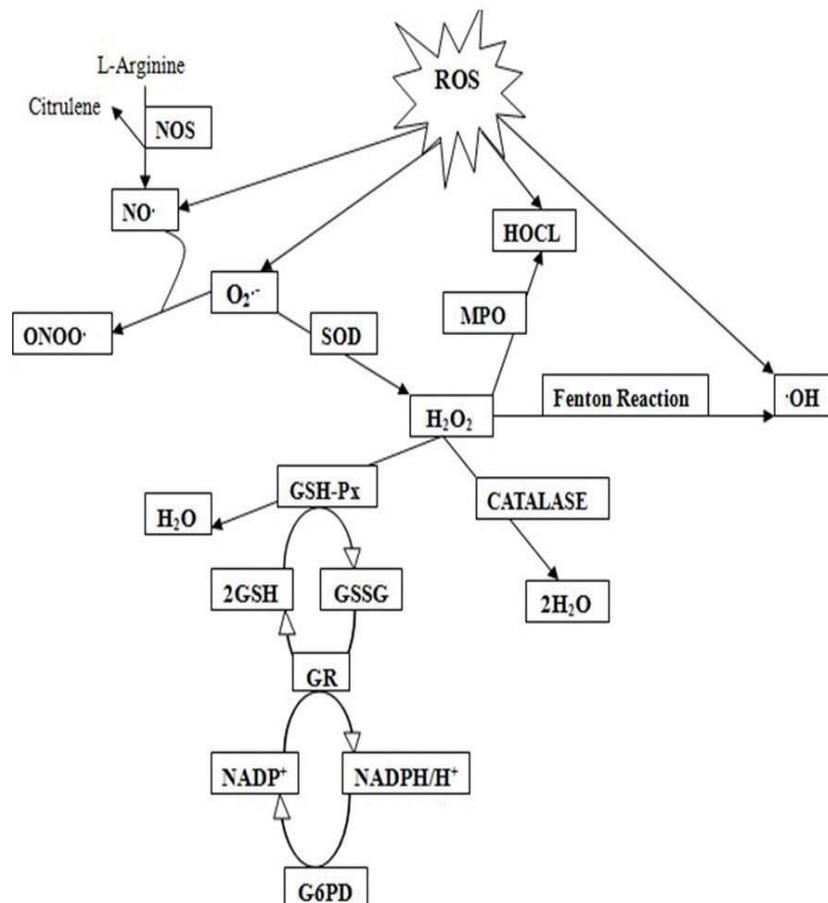


Figure 4-Shows different types of reactive oxygen species (ROS).

Abbreviations: Glutathioneperoxidase (GSH-Px), Hypochlorous acid (HOCl), Hydrogen peroxide (H₂O₂), Myeloperoxidase (MPO), Nitric oxide (NO \cdot), NO synthase (NOS), Superoxide anion (O₂ \cdot -), Hydroxyl radical (\cdot OH), Peroxynitrite anion (ONOO \cdot), Superoxide dismutase (SOD), Nicotinamide adenine diphosphate (NADPH), Reduced glutathione (GSH), Glutathione reductase (GR), Glucose-6-phosphate dehydrogenase (G6PD).

Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms. (Sies,1991) and/or from a decrease in antioxidant defense, which may lead to damage of biological macromolecules and disruption of normal metabolism and physiology .(Trevisan et al,2001). This condition can contribute and/or lead to the onset of health disorders. (Miller et al.1993), and play a damaging role in a number of liver disorders, for example, in anoxic and re oxygenation injury during transplantation, activated phagocytes and xanthine oxidase formed during ischemia, catalyze the formation of superoxide during reperfusion (Southorn & Powis, 1988;Nauta et al., 1990; Brass et al., 1991; Rosser & Gores, 1995).

Lipid peroxidation is implicated in the pathogenesis of several hepatic disorders in human(Poli, 1993; Farinati et al., 1995) and animals (Mudroň et al., 1999; Abd Ellah et al., 2004).Hepatic failure in cattle was associated with decreased antioxidant mechanisms inside the cells, which led to the increase in the reactive oxygen species, especially H₂O₂. The decreasing hepatic GSH-Px activity in severe fatty degeneration, for example, results in the increaseH₂O₂ (Abd Ellah et al., 2004), which can initiate free radical formation through Fenton'sreaction. In addition, the decrease in hepatic vitamin E level, which is an important chain breaking antioxidant, results in lipid peroxidation and failure to regenerate the ascorbic acid(Mudroň et al., 1997, 1999). Increased hepatic oxidative stress was also reported in cows suffering from glycogen degeneration (Abd Ellah et al., 2004), sawdust liver and liver abscesses (Spolarics, 1999; Abd Ellah et al., 2002; Sayed et al., 2003). The authors contended that the antioxidant defense was high in the

case of sawdust liver, glycogen degeneration, and liver abscess, which indicated that the body can combat the increased free radical stress.

Liver abscesses in fattening steers occur mainly due to intensive feeding of highly concentrated rations. Consumption of a carbohydrate-rich diet stimulates G6PD expression in endothelial and parenchymal cells (Khan et al., 1987; Spolarics, 1999). Since G6PD supports reactive oxygen metabolism, the response may represent an antioxidant pathway in the hepatic cell populations that targets sinusoid born reactive oxygen species during infections (Spolarics, 1999; Abd Ellah et al., 2002).

ANTIOXIDANTS AND IT'S ROLE IN LIVER PROTECTION.

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contribute to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction.

Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals

before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being.

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage.

ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

ANTIOXIDANT PROTECTION

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both

endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

These components include:

- Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
- Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

DIETARY ANTIOXIDANTS

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant.

ENDOGENOUS ANTIOXIDANTS

In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-

induced cell damage. The antioxidant enzymes – glutathioneperoxidase, catalase, and superoxide dismutase (SOD) – metabolizeoxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. Intensive agricultural methods have also resulted in significant depletion of these valuable trace minerals in our soils and the foods grown in them. Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cystine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism.

Free radicals are highly reactive substances produced continuously during metabolic processes. They participate mainly in physiological events such as the immune response, metabolism of unsaturated fatty acids, and inflammatory reaction. The balance between free radicals and antioxidants is disrupted in many diseases. This disruption may be attributedto a number of factors such as the inability of the cells to produce sufficient amounts of antioxidants, the nutritional deficiency of minerals or vitamins, and the excess production of reactive oxygen species. (Abd Ellah, 2010). Free radical excess results in impairment of DNA, enzymes, and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which, in turn, may be related to mutagenesis and aging processes. (Poli, 1993).The involvement of oxidative stress in the pathogenesis of hepatic dysfunction in human and animals. (Khan et al, 1987) has been investigated for many years. Some of the liver diseases were associated with increase (Farinati et al., 1995; Abd Ellah et al., 2002, 2008) or decrease (Mudroň et al., 1997; Barbaro et al., 1999; Abd Ellah et

al., 2004) antioxidants contents. Usually hepatic antioxidants increased at the beginning of hepatic disease and decreased in severe hepatic injury. The advantages of measuring hepatic oxidative status in liver are that it helps in diagnosis of hepatic dysfunction, reflects the degree of deterioration in the liver tissues, and helps to determine the severity of hepatic injury, and also, aid in recommending antioxidant's therapy in patients that had a hepatic disease with derangement in hepatic antioxidant constituents. The main purpose of the current article is to explore a tool for detection of hepatic oxidative stress. A focus was done on different types of free radicals, antioxidants, lipid per oxidation, and hepatic and blood oxidative status in hepatic dysfunction.

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3. AIM AND PLAN OF WORK

AIM OF PRESENT STUDY

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications. Looking to the scope of herbal drugs and increasing its demand especially in disease of liver, hypertension, diabetes, cancer, arthritis and skin disease etc, it was planned to study *Solanum seafortianum* in detail due its effectiveness for liver problems, especially for jaundice.

Solanum seafortianum, selected for study was made on the basis of its,

- Easy availability
- Folklore claiming its therapeutic use as liver protection and for jaundice.
- Degree of research work which is not done.

Very less pharmacological studies have been carried out on the *Solanum seafortianum*. Hence, we have decided to submit the project on *Solanum seafortianum* which includes detailed Pharmacognostical, Preliminary Phytochemical and Pharmacological (Acute oral toxicity study, Hepatoprotective activity with antioxidant effect) studies to provide scientific validation to its folklore claims.

THE PLAN OF WORK

The plan of work for the study of *Solanum seaforthianum* was carried out as follow.

1. Collection and authentication of plant material.
2. Pharmacognostical studies
 - a. Ash value
 - b. Extractive value
 - c. Loss on drying
3. Preliminary Phytochemical studies
 - a. Preparation of extract
 - b. Qualitative phytochemical studies
4. Pharmacological studies
 - a. Screening of Hepatoprotective activity,
 - Assessment of Liver Function Tests.
 - Assessment of Antioxidant parameters.
 - Histopathology of liver.

4. PLANT PROFILE



Figure.5. Images of *Solanum seaforthianum*.

GENERAL INFORMATION:

Common name	-	Brazilian nightshade, vining solanum potato creeper, St Vincent lilac
Binomial name	-	<i>Solanum seaforthianum</i> Andrews.
Family	-	Solanaceae

TAXONOMICAL CLASSIFICATION.

Domain	:	Eukaryota
Kingdom	:	Plantae
Phylum	:	Spermatophyta
Subphylum	:	Angiospermae
Class	:	Dicotyledonae
Order	:	Solanales
Family	:	Solanaceae
Genus	:	<i>Solanum</i>
Species	:	<i>Solanum seaforthianum</i>

Geographical source:

Solanum seaforthianum is an aquatic plant, belongs to the family Solanaceae, It is a floating, soloniferous herb found in ponds and streams almost throughout India. It is distributed in the tropical and subtropical region of Africa, Asia, and America.

Description:

Woody vines, twining by the petioles. Stems terete, glabrous or sparsely pubescent. Leaves simple or more often pinnatifid or pinnately lobed with up to 4 pairs of leaflets, (2-) 3.5-10(-13) x (1-)2-9(-11) cm, elliptic to broadly triangular in outline, widest in the basal third, membranous, the upper surfaces glabrous or with tiny simple uniseriate trichomes on the veins and margins, the lower surfaces glabrous; base acute, truncate or slightly cordate, occasionally oblique and asymmetric; margins less commonly entire, usually 3-7 lobed, the lobes to 5 x 2 cm, smaller basiscopically; apex acute to acuminate; petioles 1-4 cm long. Inflorescences terminal, later lateral, to 25 or more cm long, with many open, divaricate branches, with up to 100 or more flowers, glabrous; pedicels 0.8-1.4 cm, Flowers all perfect, 5-merous. Buds globose, slightly inflated, the corolla strongly exerted from the calyx tube long before anthesis. Calyx tube approximately 0.5 mm, flattened and open, the lobes < 0.2 mm long, Corolla 1.1-2.5 cm in diameter, violet or pale violet, stellate-rotate, lobed $\frac{1}{2}$ to $\frac{2}{3}$ of the way to the base, the lobes 5-9 x 3-4.5 mm, spreading or slightly cupped at an thesis, densely and minutely pubescent on the tips and margins; free portion of filaments markedly unequal, the longest filament 2-3 mm, glabrous; anthers 2-3 x 1-1.5 mm, occasionally one anther slightly larger, ellipsoid, loosely connivent, yellow, poricidal ;ovary glabrous; style 7-10 mm long, stigma capitate, minutely papillose. Fruit a globose berry, 0.8-1.4 cm in diameter, bright shiny red when ripe, glabrous, the pericarp thin; Seeds 4-20 per berry, 4-4.5 x 2.5-3 mm, flattened-reniform, pale yellowish tan, the surfaces minutely pitted (Knapp, 2010).

Chemical constituents:

Solanum seaforthianum contain alkaloids, glycosides, flavonoids, and steroids. An analysis of leaves and stems revealed the following: moisture 92.5%, protein 1.4%, fat 0.3%, carbohydrate 2.5%, fibers 0.9%, ash 1.9%, calcium 0.2%, phosphorus 0.06%. Leaves are rich in vitamin C and A, and also contain vitamin B. The ash is rich in sulfate and potassium chloride. Stigmasta-4,22-dien-3-one, stigmasterol, stigmasteryl stearate, and palmitic acids are reported in *P. stratiotes*. Plant gave 2-di-C-glycosylflavones of vicenin and lucenin type, anthocyanin-cynidin-3-glucoside, luteolin-7-glycoside and mono-C-glycosyl flavones – vitexin and orientin.

Habits:

S. seaforthianum is an ornamental vine cultivated in both tropical and subtropical climates in elevations ranging from sea levels to 1300 -1500 metres (MacKee, 1994; Flora of China Editorial Committee, 2014). Once naturalized, the species can be found growing in natural forests, natural grasslands, forest margins, urban bushland, riverbanks, crops, orchards, pastures, roadsides, disturbed sites, and waste areas in both dry and wet environment.

Medicinal uses:

The plant is considered antitubercular, antiseptic and antidysentric. In Gambia, the plant is used as an anodyne for eyewash and Juice of plant is used by Mundas in ear complaints. The ash of the plant is applied to the ringworm of the scalp. Leaves are used in eczema, ulcers, piles, leprosy and syphilis. Juice of leaves boiled in coconut oil is applied for externally in chronic skin diseases.

COLLECTION AND IDENTIFICATION

COLLECTION OF SPECIMEN

The species for the proposed study that is leaves of *Solanum seaforthianum* were collected carefully from kottayam district, Kerala.

TAXONOMICAL IDENTIFICATION:

Solanum seaforthianum is grown in the ponds and streams of some area Kerala. For the present work the plant was collected from Vikom, Kottayam district of Kerala and authenticated the plant as *Solanum seaforthianum* of family Solanaceae from available literature.

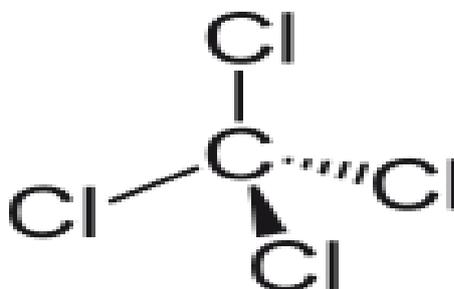
TREATMENT OF PLANT MATERIAL:

The leaves of *Solanum seaforthianum* were washed thoroughly with water to remove the mud or dust, then it was shade dried completely. The dried leaves of *Solanum seaforthianum* were then powdered by means of a mixer grinder and were sieved through sieve no. 60 to get the coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

DRUG PROFILE

CARBON TETRACHLORIDE

Chemical Structure:



Molecular formula: CCl₄

Molecular mass: 158.82 gm/mol

IUPAC Name: Tetrachloromethane

Other names: Benziform , Carbon Tet , Tetrazol

Appearance: Colourless liquid

Odor: ether like odor.

Density: 1.5867 gm/cm³

Melting point: -22.92° C

Boiling point: 76.72° C

Solubility: Soluble in alcohol, ether, CHCl₃, Benzene.

USES:

It was widely used as a dry refrigerant, cleaning solvent, in detection of Neutrinos. It is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents.

SILYMARIN (reference drug):

A mixture of the isomers Silibinin, Silicrystin and Silidianin and the active principle from the fruit of *Silybummarianum* (*Carduusmarianus*) (Compositae).The principle components are the Silibinin, silicrystin, silidianin and flavonolignans of which Silibinin is the major component.

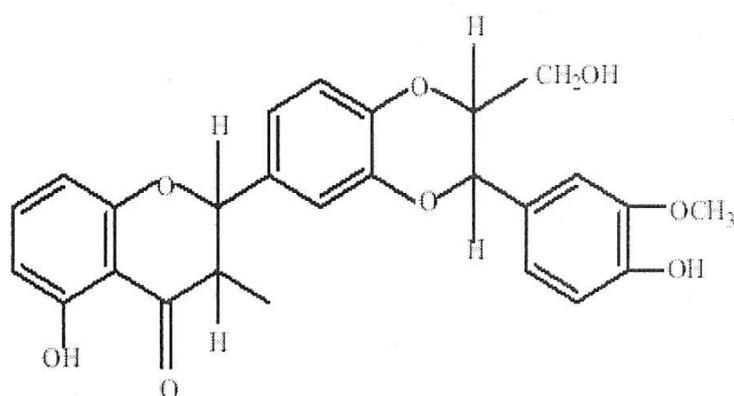
Chemical Structure of Silymarin**PICTURE OF SILYMARIN:**

Figure 6: Picture of Silymarin

Chemical Name : 2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl) -
2(hydroxymethyl)-6-(3,5,7-trihydroxy-4-oxobenzopyran-2-yl) benzodioxin.

Molecular Formula : $C_{25}H_{22}O_{10}$

Molecular Weight : 482.44

Scientific name : Milk Thistle

Other Names: Carduimariae, Mary Thistle, Holy Thistle, Lady's Thistle, Marian Thistle, Mariendistel, Our Lady's Thistle, Silimarina, Carduusmarianum, Silybin, Silybummarianum, St. Mary Thistle. , Legalon

PROPERTIES:

Hepato-protective: Protects liver cells from incoming toxins, thereby also allowing it to more effectively process and release toxins that are already built up in system.

Hepato-tonic: Strengthen the liver to function more effectively.

Anti-depressant: By helping to move stagnant liver energy.

Demulcent: Soothes and moistens bladder and kidneys irritations, as well as mucous membranes and inflammations of the skin and integumentary system.

Laxative: Mildly lubricates the bowels.

Hepatoprotective role of silymarin:

Silymarin has shown differing degrees of effectiveness for protecting the liver from damage caused by some drugs, alcohol, chemicals, diseases, and poisonous plants. It is used to treat both acute conditions (such as poisoning) and long term diseases (such as Hepatitis C).

Silymarin and other chemicals in milk thistle are believed to protect liver cells in several different ways.

Silymarin has antioxidant properties/free radical scavenging effect and has been used for the treatment of liver disorders.

Anti-inflammatory effects of Silymarin help in preventing hepatic cells from swelling after being injured.

Silymarin seems to encourage the liver to grow new cells, while discouraging the formation of inactive fibrous tissue.

By changing the outside layer of liver cells, Silymarin actually may keep away certain harmful chemicals from getting into liver cells.

Milk thistle may also cause the immune system to be more active.

5. MATERIALS AND METHODS

PHARMACOGNOSTICAL STUDIES:

Ash values, extractive value, loss on drying are used for the study of physical properties.

ANALYTICAL PARAMETERS

ASH VALUES

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form.

The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. There is a considerable difference varies within narrow limits in the case of the same individual drug. Hence an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of official drugs. Usually these standards get a maximum limit on the total ash or on the acid insoluble ash permitted.

The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.

The ash or residue yielded by an organic chemical compound is as a rule, a measure of the amount of inorganic matters present as impurity. In most cases, the inorganic matter is present in small amounts which are

difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drugs in powder form.

Procedures given in Indian pharmacopoeia were used to determine the different ash values such as total ash and acid insoluble ash.

Determination of total ash value:

Weighed accurately about 3 gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled and weighed and then calculated the percentage of total ash with reference to the air dried drug.

Determination of acid insoluble ash value:

The ash obtained as directed under total ash above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of Water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

Determination of sulphated ash value:

About 3 gms of accurately weighed air dried powdered drug was taken in a tared silica crucible which was previously ignited and weighed. Then ignite gently at first until the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1 ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at $800 \pm 25^\circ \text{C}$ until the black particles has disappeared. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight. The percentage of sulphated ash was calculated with reference to the air dried drug. All the ash values were calculated and recorded.

EXTRACTIVE VALUES

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value

5 gm of the air-dried coarse powder of *Solanum seafortianum* was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The

percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug. The results are recorded in the table.

Determination of water soluble extractive value

Weigh accurately 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug. The results are given in the table.

LOSS ON DRYING

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Desiccators or hot air oven). If the sample is in the form of large crystals, then reduce the size by quick crushing to a powder.

Procedure

About 1.5 gm of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of leaf of *Solanum seaforthianum*:

About 200 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days to remove fatty substances. At the end of 2nd day the powder was taken out and it was dried. After drying it was again packed and extracted by using methanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness.

Table: 1. Nature and colour of ethanol extract of *Solanum seaforthianum*.

S.No.	Name of extract	Colour	Consistency	Yield% W/W
1	Methanolic extract	Dark greenish	Sticky mass	12.4

CHEMICAL TESTS:

1) Test for carbohydrates

1. Molisch Test: It consists of treating the compounds of α -naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al*, 2000)

2. **Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.
3. **Benedict's test:** To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.
4. **Barfoed's test:** To the 5ml of the Barfoed's solution add 0.5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

2) Test for Alkaloids

1. **Dragendorff's Test:** To the extract, add 1ml of Dragendorff's reagent. Orange red precipitate is produced.
2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate is produced.
3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.
4. **Hager's Test:** To the extract add 3ml of Hager's reagent. Yellow precipitate is produced.

3) Test for Steroids and Sterols

1. **Liebermann Burchard test:** Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.
2. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red

and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

4) Test for Glycosides

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.
2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.
3. **Bortrager test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.
4. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

5) Test for Saponins

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

6) Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red colour is produced.

7) Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

8) Tests for Tannins and Phenolic Compounds:

The Phenol content in the raw material of *Solanum seaforthianum* extract was estimated by spectroscopically method.

To 2-3 ml of extract, add few drops of following reagents:

- a) **5% FeCl₃ solution:** deep blue-black color.
- b) **Lead acetate solution:** white precipitate.
- c) **Gelatin solution:** white precipitate
- d) **Bromine water:** decolouration of bromine water.
- e) **Acetic acid solution:** red color solution
- f) **Dilute iodine solution:** transient red color.
- g) **Dilute HNO₃:** reddish to yellow color.

9) Test for Fixed Oils and Fatty acids**a). Spot test:**

Small quantity of the extract is placed between two filter papers. Oil stain produced with any extract shows the presence of fixed oils and fats in the extracts.

b). Saponification test:

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated

on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

10) Test for Gums and Mucilage:

a) Ruthenium red test:

Small quantities of extract are diluted with water and added with ruthenium red solution. A pink colour production shows the presence of gums and mucilage.

11) Test for Proteins and Amino acids

Biuret test: Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

Ninhydrin test: Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

PHARMACOLOGICAL EVALUATION

Animals:

Healthy male Wistar albino rats of 2 to 3 months of age and approximately weighing between 150-250g were used in the present study. Rats were housed in a polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature $25\pm 2^{\circ}\text{C}$.

All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethics committee, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal. (Regd. No. JKKMMRFCP / 1158 / PO / ac / 07CPCSEA)

EXPERIMENTAL PROTOCOL:

The rats were divided into five groups, comprising of six animals in each group.

Group – I: Received 5% CMC 10ml/kg body weight. The group served as a normal control.

Group – II: Received CCl₄ (0.7 ml/kg) body weight intraperitoneally.

Group - III: Received Silymarin, the standard drug (25mg/kg).

Group – IV: Received methanolic extract of leaves of *Solanum seaforthianum* 100mg/Kg.

Group – V: Received methanolic extract of leaves of *Solanum seaforthianum* 200mg/Kg.

0.7 ml/kg of CCl₄ was injected intra peritoneally (i.p.) to all groups except normal control to induce hepatotoxicity on 3, 6 and 10thdays of experiment. Duration of the study was 10 days. All the animals were sacrificed on the 11th day for the estimation of biochemical parameters. (Prakash yoganandam.G. et.al.2009).

Estimation of serum biochemical parameters:

On 11th day blood was collected from animals under anaesthesia by cardiac puncture. Blood samples collected was centrifuged at 3500 rpm for

15 mins at room temperature for separation of serum. The clear, non-haemolysed sera was separated using clean dry disposable plastic syringe and stored at -20°C for measurements of the following,

Liver function test:

- Serum glutamic pyruvic transaminase (SGPT)
- Serum glutamic oxaloacetic transaminase (SGOT)
- Alkaline phosphatase (ALP)
- Total protein (TP)
- Total bilirubin (TB)

Lipid profiles:

- Cholesterol
- Triglycerides (TG)
- High Density Lipoprotein (HDL)
- Low Density Lipoprotein (LDL) and
- Very Low Density Lipoprotein (VLDL)

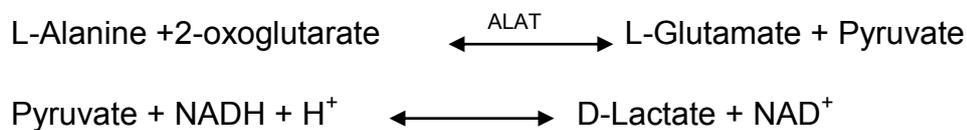
EVALUATION OF SERUM PARAMETERS:

All the above biochemical parameters were estimated using serum. All the above biochemical parameters were estimated using semi-auto analyzer (Photometer 5010 v5+, Germany) with enzymatic kits procured from Primal Healthcare limited, Lab Diagnostic Division, and Mumbai, India.

1) ESTIMATION OF SGPT

Serum Glutamate Pyruvate Transaminase (SGPT)

Principle



Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, eg., from patients with myocardial infarction, liver diseases and intensive care patients.

Method

Kinetic UV test, according to the international federation of clinical chemistry and laboratory medicine (IFCC)

Table 2: Shows reagents of SGPT in the kit

Reagent-1	Concentration
TRIS PH 7.5	100 mmol/l
L-Alanine	500 mmol/l
LDH (lactate dehydrogenase)	= 1200 U/l
Reagent-2	Concentration
2-Oxoglutarate	15 mmol/l
NADH	0.18 mmol/l
Good's buffer PH 9.6	0.7mmol/l
Pyridoxal -5-Phosphate	0.09 mmol/l

Assay procedure

Mix 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube

- a) To this, added 100 µl of serum.
- b) Mixed well and took the reading immediately. Normal range: <41 u/l

2) ESTIMATION OF SGOT

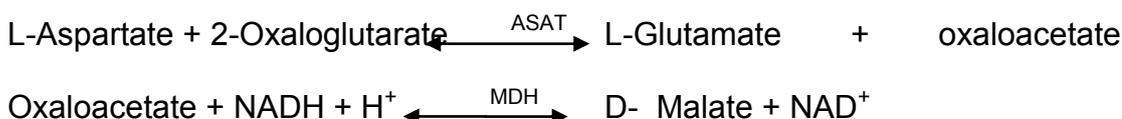
Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle

Alanine aminotransferase (ALAT) and aspartate amino transferase (ASAT) are the most important of a group of enzymes of aminotransferase. These enzymes act as catalyst in conversion of α-keto acids into amino acids by transfer of amino groups.

Increased levels of ALAT is found in the hepatobiliary disease condition where as increased ASAT levels occur in damaged conditions of heart and skeletal muscles well as liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages

The ASAT/ALAT ratio is used from differential diagnosis of liver diseases



Method

Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)

Table 3: shows Reagents of SGOT in the kit

Reagent-1	Concentration
TRIS Ph 7.8	80 mmol/l
L- Aspartate	240 mmol/l
MDH(malate dehydrogenase)	=600 U/l
LDH(lactate dehydrogenase)	=600 U/l
Reagent-2	concentration
2-Oxaloglutarate	12 mmol
NADH	0.18 mmol
Good buffer pH 9.6	0.7 mmol/l
Pyridoxal-5-Phosphate	0.09 mmol/l

Assay procedure

- a. Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube
- b. To this, added 100 µl of serum
- c. Mixed well and took the reading immediately

Normal range : <37u/l

3) ESTIMATION OF ALKALINE PHOSPHATASE

Principle

Alkaline phosphatase (ALP), hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver



Method

Kinetic photometric test, according to the international Federation of clinical chemistry and laboratory Medicine (IFCC).

Table 4: shows reagents of alkaline phosphatase in the kit

Reagent 1 :	Concentration
2-Amino-2-methyl-1-propanol pH 10.4	0.35 mol/l
Magnesium sulphate	2.0 mmol/l
Zinc sulphate	1.0 mmol/l
HEDTA	2.0 mmol/l
Reagent 2 :	Concentration
p-Nitrophenylphosphate	16.0 mmol/l

Test Procedure

- Take 1000 μ l of reagent-1 in a 5 ml test tube
- To this add 250 μ l of reagent-2 and mix well
- Add 20 μ l of serum and mix well and take reading immediately and read absorbance at 405nm

Normal range: 53-128 u/l

4) ESTIMATION OF TOTAL PROTEIN

Principle

Protein forms a coloured complex with cupric ions in alkaline medium

Table 5: Shows reagents of total protein in the kit

Reagent-1	Concentration
Cupric sulphate	6 mmol/l
Potassium iodide	15 mmol/l
Reagent-2	Concentration
Protein (std)	6 g/100ml

Method: Biuret method

Procedure:

Preparation of test sample

Take 1 ml of reagent-1 in a 5 ml test tube

To this add 0.02 ml of serum

Mix well and incubate at a room temperature for 15 min and Read the test sample at 750 nm. Normal range: 3.2 to 4.2 g/dl

5) ESTIMATION OF CHOLESTEROL

Cholesterol was estimated by the following method.

Table 6: Shows reagents of cholesterol in the kit

Reagent	Concentration
Ferric chloride-acetic acid reagent	0.05%
Cholesterol stock standard	1 mg/ml in acetic acid
Cholesterol working standard	40 µg in ferric chloride-acetic acid reagent
Concentrated sulfuric acid.	3.0 ml

Procedure

0.1 ml of extract was evaporated to dryness and 5.0 ml ferric chloride-acetic acid reagent was added, mixed and centrifuged. To the supernatant 3.0 ml of concentrated sulfuric acid was added and the absorbance was read after 20 min at 560 nm against a reagent blank. A Standard in the concentration range of 40-200 µg was treated similarly.

Values were expressed as mg/dL serum.

6) DETERMINATION OF TRIGLYCERIDES

Triglycerides were determined by the following method.

Triglycerides are extracted by isopropanol, which upon saponification with potassium hydroxide yield glycerol and soap. The glycerol liberated is treated with meta per iodate, which releases formaldehyde, formic acid and iodide. The formaldehyde released reacts with acetyl acetone and ammonia forming yellow coloured compound, the intensity of which is measured at 420nm.

Table 7: Shows reagents of triglycerides in the kit

Reagent	Concentration
Saponification reagent	0.6 ml
Sodium meta per iodate reagent	650 mg.
Acetyl acetone reagent	0.5 ml
Standard triolein solution	1 g of triolein was dissolved in 100 ml isopropanol

Reagents:

1. Isopropanol
2. Activated aluminium oxide (Neutral)
3. Saponification reagent – 5 g of potassium hydroxide was dissolved in 60 ml of distilled water and 40 ml of isopropanol was added to it
4. Sodium meta per iodate reagent - 77 g of anhydrous ammonium acetate was dissolved in about 700 ml of distilled water, 60 ml glacial

acetic acid was added to it followed by 650 mg of sodium meta periodate. The mixture was diluted to 1 litre with distilled water

5. Acetyl acetone reagent - 0.75 ml of acetyl acetone was dissolved in 60 ml of distilled water and 40 ml of isopropanol was added to it
6. Standard triolein solution - 1 g of triolein was dissolved in 100 ml isopropanol. 1 ml of stock standard was diluted to 100 ml to prepare a working standard 100 µg of triolein/ml.

Procedure

An aliquot of serum/lipid extract was evaporated to dryness. 0.1 ml of methanol was added followed by 4 ml of isopropanol. 0.4 g of alumina was added to all the tubes and shaken well for 15 min. Centrifuged and then 2 ml of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65°C for 15 min for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40 µg triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm.

The triglyceride content was expressed as mg/dL - serum

7) ESTIMATION OF HDL-CHOLESTEROL:

Principle:

The common classification of lipoproteins – HDL, LDL and VLDL comes mainly from ultra centrifugation of serum or plasma. HDL (specific gravity more than 1.063) can be separated by using polyionic substances

along with bivalent metal ions. HDL is separated from other protein fractions by treating serum with phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated. Centrifugation separates high density lipoproteins as a clear supernatant. Cholesterol content of which is estimated by enzymatic method as described earlier in estimation of total cholesterol.

Reagents:

1. Enzyme reagent
2. Diluent buffer
3. Precipitating reagent PEG-6000
4. Standard – 200 mg/dL

The working reagent is prepared by dissolving enzyme reagent with 25ml of diluent buffer and kept for at least 10 min before use. The working reagent is stable for 4 weeks at 2-8°C.

Procedure:

0.5 ml of serum was taken into a glass tube and added 0.05 ml of precipitating reagent. Mixed well, and kept at room temperature for 10 min. the mixture was then centrifuged for 15 min at 4000 rpm. Separated the clear supernatant and immediately determined cholesterol content.

8) ESTIMATION OF LDL-CHOLESTEROL AND VLDL-CHOLESTEROL:

The amounts of LDL-cholesterol and VLDL-cholesterol were calculated by using the Friedwald formula.

$$\text{VLDL-Cholesterol} = \text{Triglycerides} / 5$$

$$\text{LDL-Cholesterol} = \text{Total-Cholesterol} - (\text{HDL} + \text{VLDL})$$

The levels of HDL, LDL and VLDL-cholesterol are expressed as mg/dL.

Estimation of antioxidant parameters:

On 11th day animals were sacrificed after blood withdrawal and abdomen was cut open and liver was dissected out.

The liver was perfused with 0.86% cold saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized using a homogenizer. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris. The supernatant was used for the estimation of Catalase, Superoxide dismutase, Glutathione peroxidase and Lipid peroxidase.

INVIVO ANTIOXIDANT ENZYME ESTIMATION

The homogenate was centrifuged and supernatant was used for the assay of marker enzymes namely,

Catalase (CAT)

Superoxide dismutase (SOD)

Lipid peroxidation (LPO)

1. ESTIMATION OF CATALASE (CAT) ACTIVITY

Reagents:

Dichromate/acetic acid reagent (5% solution of potassium dichromate in acetic acid at 1:3 ratios)

0.01 M Phosphate buffer, pH 7.0

0.2 M Hydrogen peroxide

Procedure:

Catalase(CAT) was estimated by the method of Sinha (1972).The reaction mixture (1.5ml vol) contained 1.0 ml of 0.01 M phosphate buffer (Ph7.0) 0.1 ml of tissue homogenate and 0.4 ml of 2M H₂O₂. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).Then the absorbance was measured at 530 nm;

2. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Reagents

1. Adrenaline
2. Carbonate buffer (pH 10.2)
3. Mm EDTA

Procedure:

The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar et al based on the oxidation of epinephrine adrenochrome transition by enzyme. The post-mitochondrial suspension of mice liver (0.5ml)

was diluted with distilled water (0.5). To this chilled ethanol (0.25ml) and chloroform (0.15ml) was added. The mixture was shaken for 1 min and centrifuged at $2000 \times g$ for 10 min. The PMS (0.5ml) was added with PBS buffer (Ph 7.2; 1.5ml). The reaction initiated by the addition of epinephrine (0.4ml) and change in optical density (O.D., min⁻¹) was measured at 470 nm. SOD activity was expressed as U/l of tissue. Change in O.D (min⁻¹) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

3. ESTIMATION OF LIPID PEROXIDATION OF RAT LIVER:

Reagents

1. Thiobarbituric acid 0.37%
2. 0.25 NHC1
3. 15% TCA

Procedure:

Lipid peroxidation in liver was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) using the method of Fraga et al. (1988). In brief, 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid -HC1 reagent (0.37% TBA, 0.25 M HC1 and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at $3500 \times g$ for 10 min at room temperature, the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were observed.

HISTOPATHOLOGICAL INVESTIGATIONS

A portion of liver processed for histopathological investigations.

Histopathological techniques

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of materials

Thin pieces of 3 to 5 mm, thickness are collected from tissues showing gross morbid changes along with normal tissue.

Fixation

Keeping the tissue in fixative for 24-48 hours at room temperature

1. Serves to harden the tissues by coagulating the cell protein,
2. Prevents autolysis.
3. Preserves the structure of the tissue, and
4. Prevents shrinkage: The volume of the fixative added is 10 times the volume of the tissues. Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol, then wash in tap water. Stain with haematoxylin for 3-4 minutes and wash in tap water. Allow the sections in tap water 5-10 min and wash in tap water. Counter stain with 0.5% until section appears light pink (15 to 30seconds), and then wash in tap water. Blot and dehydrate in alcohol.

Clear with xylol (15 to 30 seconds). Mount in Canada balsam or DPX Moutant. Keep slide dry and remove air bubbles.

STATISTICAL ANALYSIS

All the values of biochemical and antioxidant parameter estimations were expressed as mean \pm standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test, using InStat v.2.02 software (GraphPad Software Inc.). Differences between groups (p Value) were considered significant at $P < 0.05$.

6. RESULTS AND DISCUSSION

The fern, *Solanum seaforthianum* belonging to family solanaceae was selected for my project, on the basis of ethanobotanical information which reveals its uses against one of the most hazardous disease.

Solanum seaforthianum, is commonly called as Brazilian nightshade, solanum potato creeper, St Vincent lilac herb found in almost throughout India.

Literature survey revealed that not much work has been done on this fern claiming maximum therapeutic uses. So we felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed Pharmacognostical and preliminary phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

1. PHARMACOGNOSTICAL STUDIES:

ANALYTICAL PARAMETERS

The analytical parameters were investigated and reported as, total ash value (9.1 %w/w), water soluble ash value (3.5%w/w), acid insoluble ash value (3.4 %w/w),, sulphated ash value (5.2 %w/w), water soluble extractive value (3.7 %w/w), alcohol soluble extractive value (4.5 %w/w), loss on drying (5.12 %w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies.

The results were given in table.

a. Ash values

Table 8

Data for ash values for powdered leaves of *Solanum seaforthianum*.

S.No	PARAMETER	%w/w
	ASH VALUES	
1.	Total Ash	9.1
2.	Water Soluble Ash	3.5
3.	Acid Insoluble Ash	3.4
4.	Sulphated Ash	5.2

b. Extractive values and loss on drying

Table 9

Data for extractive values and loss on drying of powdered leaves of *Solanum seaforthianum*.

Analytical parameter	Percentage (w/w)
Water soluble extractive	3.7 %
Alcohol soluble extractive	4.5 %
Loss on drying	5.12 %

2. PRELIMINARY PHYTOCHEMICAL STUDIES:

The leaves of *Solanum seaforthianum* were subjected for hot continuous extraction using methanol as solvent. The yield was found to be 5.27% w/w. the extracts obtained were subjected to various phytochemical tests, to identify the active constituents, which showed the presence of, alkaloids, glycosides, flavonoids, and steroids, carbohydrate, fatty acids and proteins and phenolic compounds. The results were given in table: 3.

Table 10

Results of Phytochemical analysis of powdered leaves of *Solanum seaforthianum*.

PHYTOCONSTITUENTS	ETHANOL EXTRACT
Alkaloids	+
Saponins	-
Glycosides	+
Carbohydrates	+
Tannins	-
Flavanoids	+
Terpenoids	+
Steroids	+
Phenolic compounds	+
Proteins and amino acids	+
Fixed oils and fatty acids	+
Gums and mucilage	-

3. PHARMACOLOGICAL STUDIES:

EVALUATION OF HEPATOPROTECTIVE ACTIVITY:

Assessment of serum biochemical parameters:

Methanol extract of *Solanum seaforthianum* possessed a good hepatoprotective activity on rats as shown in the table. At varying dose levels, (100 mg/kg and 200 mg/kg), this extract attenuated altered biochemical parameters produced by CCl₄ was dose dependent. This extract possessed significant hepatoprotective activity at both 100 and 200 mg/kg dose level.

Table 11

Effect of *Solanum seaforthianum* extract on serum parameters in CCl₄ induced hepatic damage in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TOTAL BILIRUBIN (mg/dl)	TOTAL PROTEIN (mg/dl)
GROUP I	97.00 ± 1.41	82.73 ± 1.47	142.00 ± 1.42	0.66 ± 0.01	10.06 ± 0.04
GROUP II	238.5 ± 1.50	363.00 ± 0.89	397.70 ± 0.75	2.14 ± 0.03	6.73 ± 0.08
GROUP III	117.56 ± 1.86	106.73 ± 0.98	166.00 ± 1.41	0.76 ± 0.02	9.48 ± 0.17
GROUP IV	204.40 ± 1.37	209.0 ± 1.42	215.00 ± 1.40	1.05 ± 0.04	8.41 ± 0.07
GROUP V	102.20 ± 1.03	105.06 ± 0.98	159.00 ± 1.42	0.79 ± 0.05	8.05 ± 3.51

Values are given as mean \pm Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Group II compared with group I and Groups III, IV & V were compared with group II.

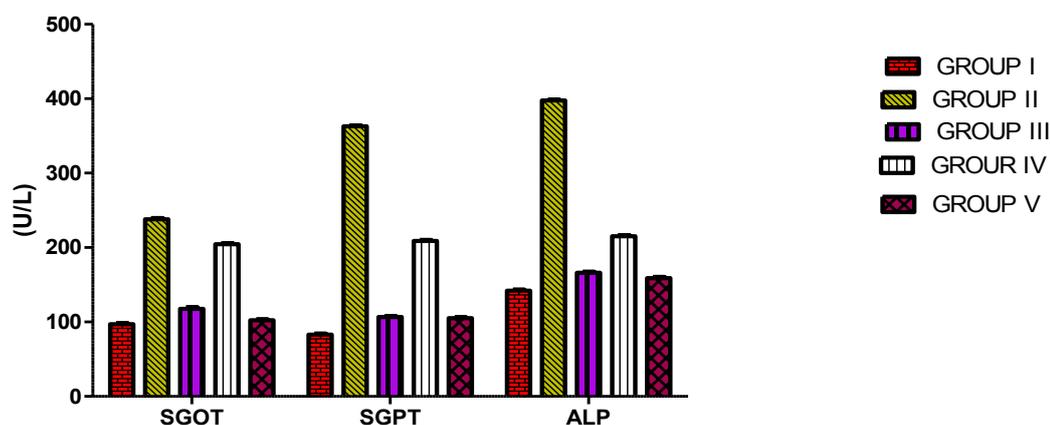


Figure 7: Effect of *Solanum seforthianum* extract on serum parameters in CCl_4 induced hepatic damage in rats

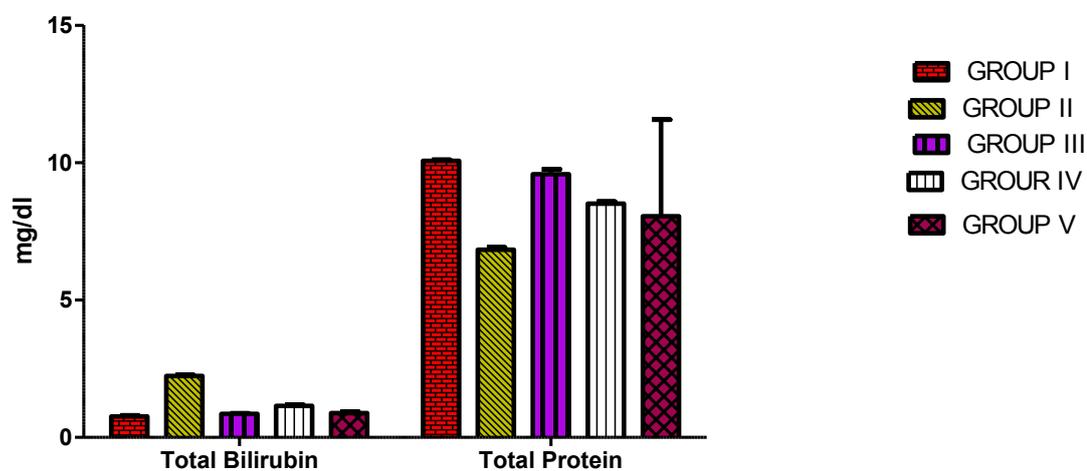


Figure 8: Effect of *Solanum seforthianum* extract on total protein and total bilirubin in CCl_4 induced hepatic damage in rats

Assessment of Lipid profiles:

The serum lipid profile such as total cholesterol, triglycerides, LDL & VLDL were elevated, and this indicated deterioration in hepatic function due to the damage caused by CCl₄ administration.

Whereas treatment of *Solanum seforthianum* extract significantly declined the effect of CCl₄ induced damage and it was evidenced by the decreased level of total cholesterol, triglycerides, LDL & VLDL and increased level of HDL in extract group.

Results were shown in table 5.

Table 12

Effect of *Solanum seforthianum* extract on liver lipid profiles in CCl₄ induced hepatic damage in rats

Treatment	CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
GROUP I	72.73 ± 1.60	80.73 ± 1.47	39.00± 1.41	31.40 ± 1.51	14.00 ± 0.89
GROUP II	126.50 ± 1.21	125.23 ± 0.81	13.23 ± 1.03	74.20 ± 0.51	36.56 ±1.36
GROUP III	8372 ± 0.75	87.23 ±1.03	29.00± 0.89	45.73± 0.75	17.56 ± 0.51
GROUP IV	95.00 ± 0.89	93.00 ± 0.89	25.73 ± 1.16	48.73 ± 1.32	21.73 ± 1.16
GROUP V	68.23 ± 1.03	83.4 ± 1.51	51.73 ± 0.75	38.00 ±1.41	14.4 ± 1.04

Values are given as mean \pm Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Group II compared with group I and Groups III, IV & V were compared with group II.

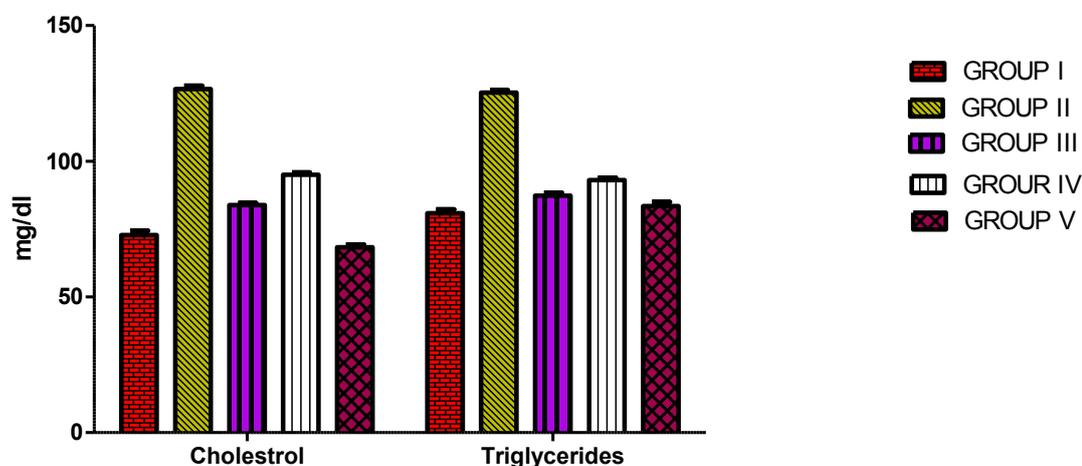


Figure 9 : Effect of *Solanum seaforthianum* extract on liver lipid profiles in CCl_4 induced hepatic damage in rats

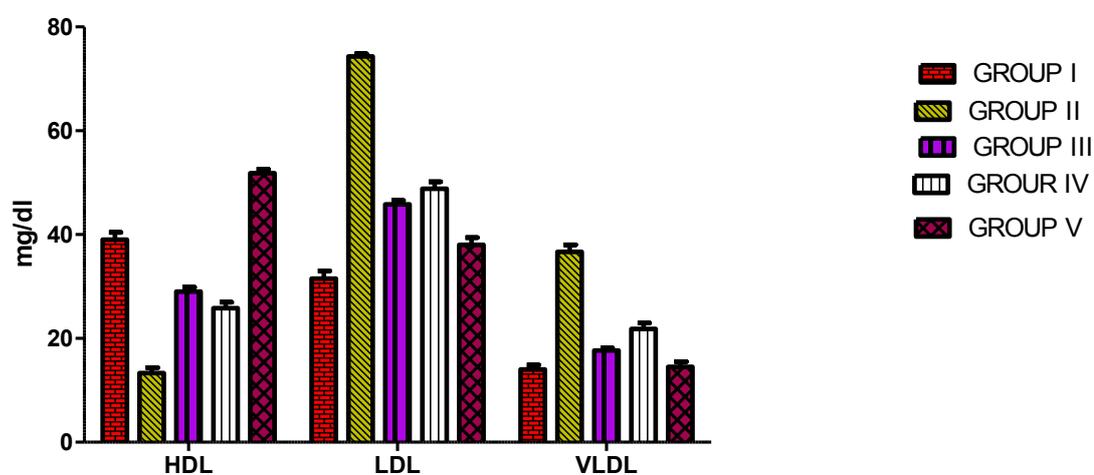


Figure 10 : Effect of *Solanum seaforthianum* extract on HDL, LDL, VLDL in CCl_4 induced hepatic damage in rats

Assessment of antioxidant parameters:

The effects of extract at two dose levels (100 and 200 mg/kg, p.o.) on liver antioxidant enzymes in CCl₄-induced hepatic injury are shown in Table 2. Hepatic injury induced by CCl₄ caused significant increases in liver antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidase. Administration of MEAI at different dose levels shows significant dose-dependent decreases, when compared with diseased control animals. Results were shown in table 6.

Table 13

Effect of *Solanum seafortianum* extract on antioxidant parameters in CCl₄ induced hepatic damage in rats

Treatment	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Lipid peroxidase (nmol/mg of protein)
GROUP I	291.56 ± 1.36	92.40 ± 1.51	4.56 ± 0.51
GROUP II	237.23 ± 0.81	81.73 ± 1.16	8.40 ± 0.54
GROUP III	287.06 ± 0.98	76.00 ± 1.26	2.73 ± 0.40
GROUP IV	195.73 ± 0.75	67.06 ± 0.75	4.56 ± 0.51
GROUP V	277.73 ± 1.16	74.24 ± 1.36	3.56 ± 0.51

Values are given as mean \pm Standard error mean (S.E.M) for five groups of six animals each. Values are statistically significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Group II compared with group I and Groups III, IV & V were compared with group

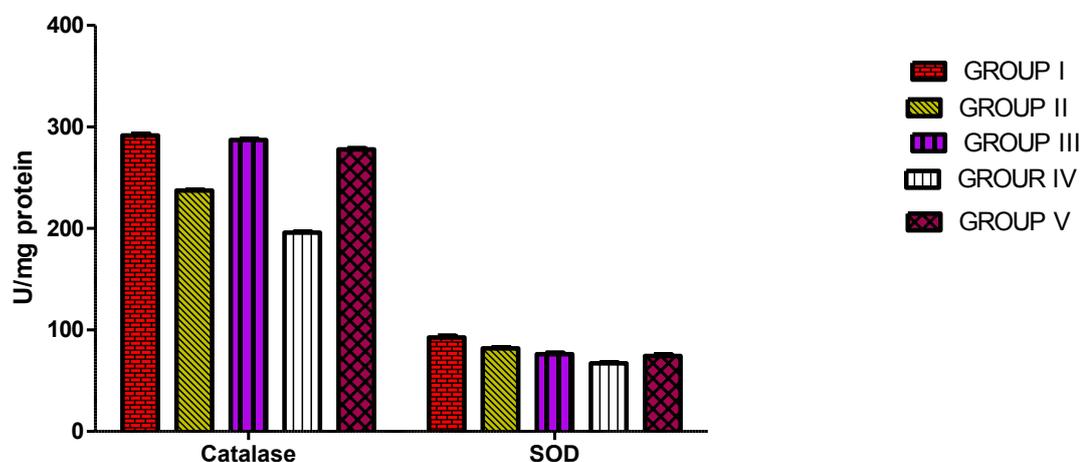


Figure 11: Effect of *Solanum seforthianum* extract on Catalase and SOD in CCl₄ induced hepatic damage in rats

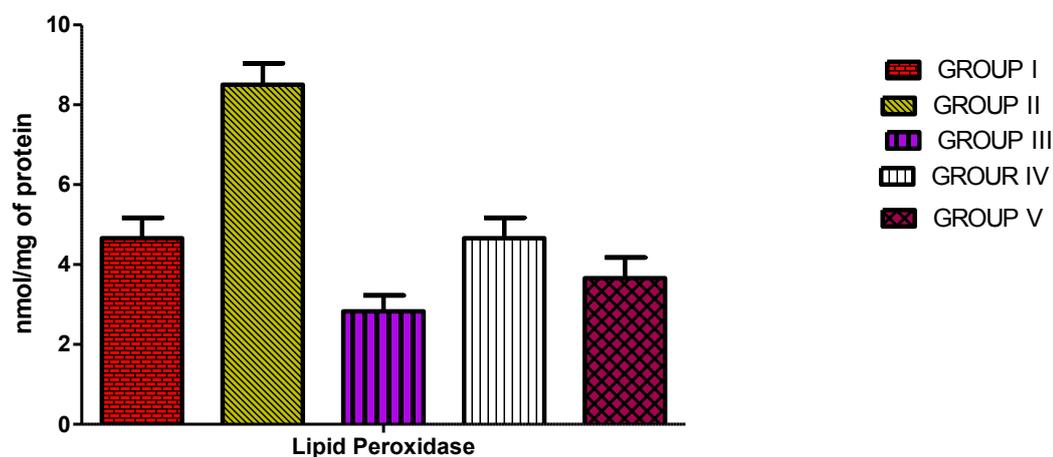
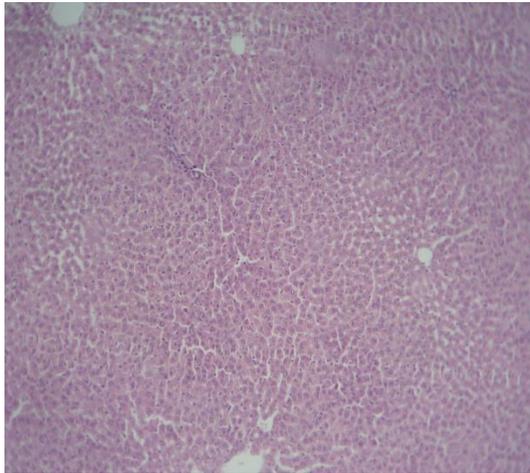
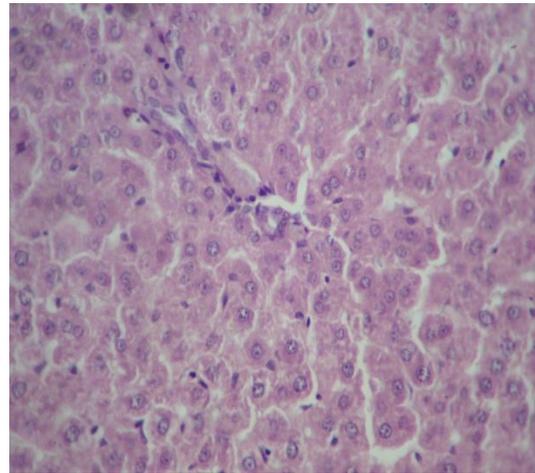


Figure 12: Effect of *Solanum seforthianum* extract on LPO in CCl₄ induced hepatic damage in rats

Group I

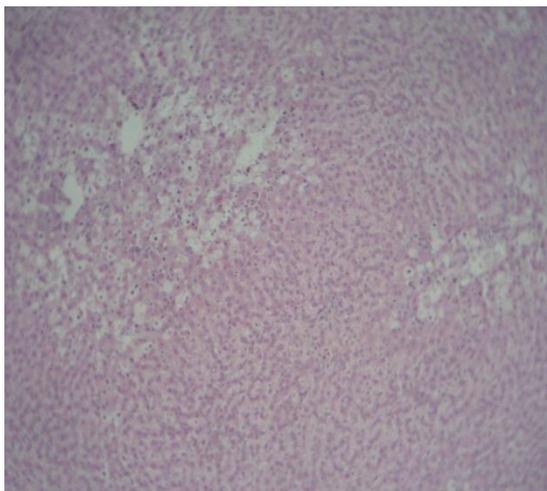


10x Liver with normal lobular architecture

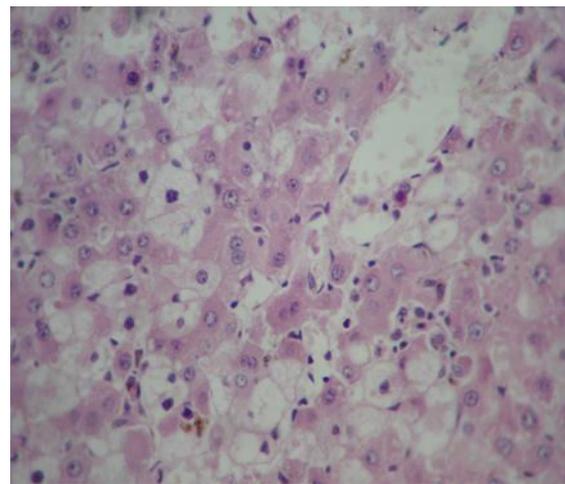


40x Normal portal tract and hepatocytes

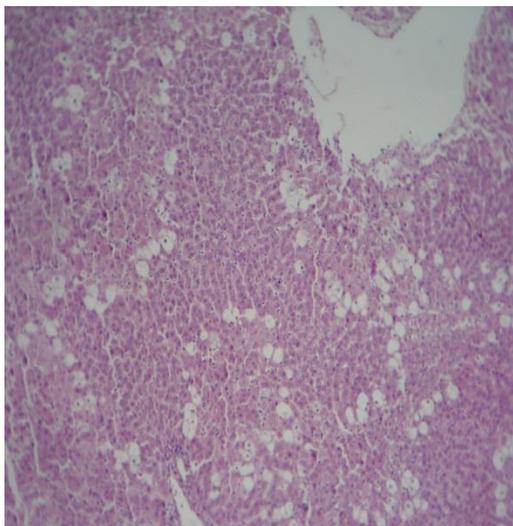
Group II



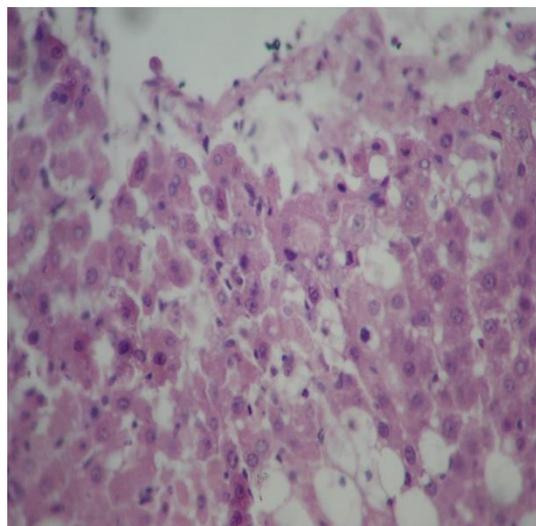
10x Liver showing parenchyma with fatty change (++++), and lobular and lobular inflammation



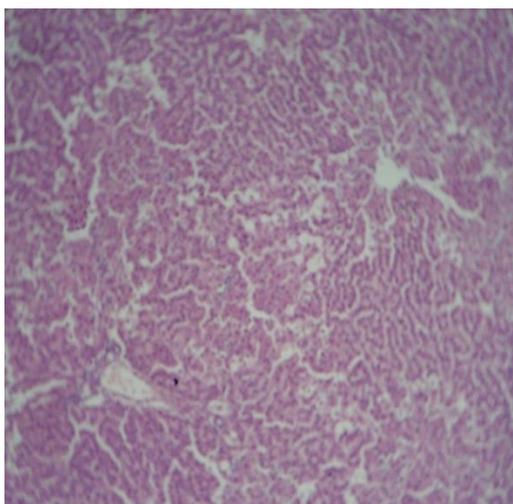
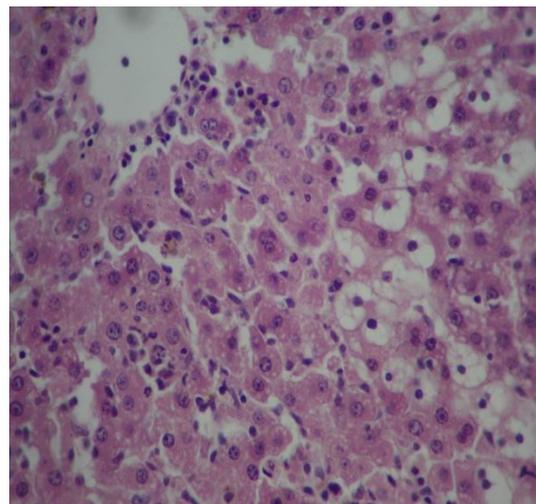
40x Perivenular region showing fatty change and inflammation

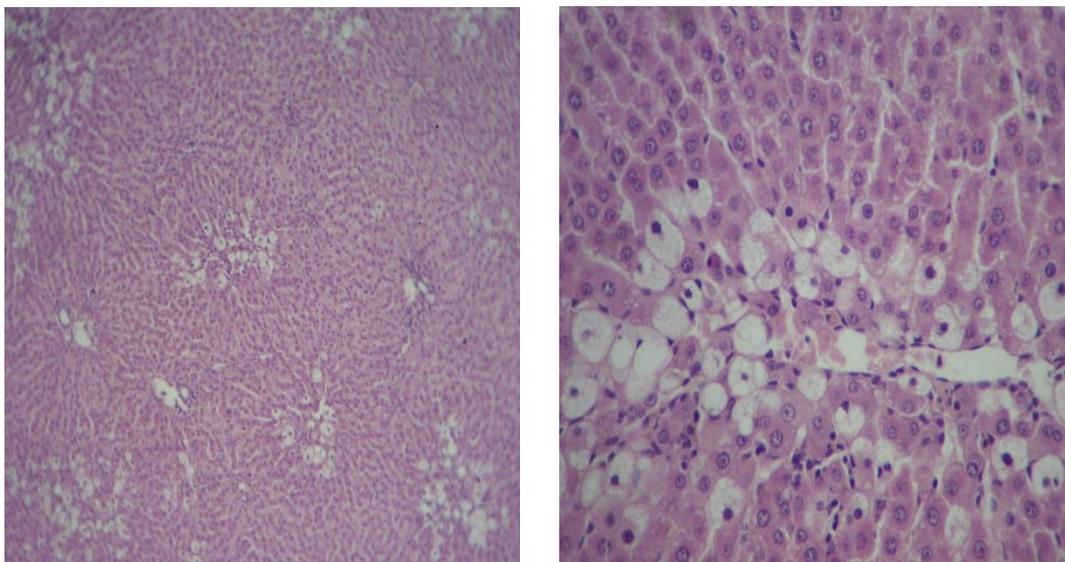
Group III

10x Liver showing parenchyma with fatty change(+)



40x Perivenular region showing fatty change.

Group IV10x Liver showing parenchyma with fatty change (+++)
and lobular inflammation40x Perivenular region showing fatty change(+++)
and inflammation

Group V

10x Liver showing parenchyma with
fatty change(+)

40x Perivenular region showing
fatty change(+)

Figure 13. HISTOPATHOLOGY OF LIVER OF RAT

Photomicrograph of liver shows a normal hepatic cellular arrangements in Group 1 (a) whereas, in group II (b) showing loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, crowding of central vein. In rats treated with silymarin (c), a normal hepatic architecture with moderate mild degree of necrosis. Group IV and group V (d & e) reduces the hepatic injury score of fatty degeneration and necrosis, clearly indicating the protection offered by MEAI.

DESCRIPTION OF HISTOPATHOLOGICAL EXAMINATION**Group -I**

Group I rat Liver tissue section shows normal lobular architecture of liver cells. Hepatocytes, hepatic sinusoids, portal tract, show normal size & Shape. The central vein and sinusoids are normal. There are no inflammation fatty change

Group - II

Group II rat Liver tissue section shows extreme degeneration of hepatic architecture by necrosis, foci of hemorrhage, fatty changes, crowding of vein. The perivenular region shows extensive fatty changes with the areas of lobular inflammation with hepatocytes (fatty change++++,lobular inflammation ++)

Group -III

Group III Liver Tissue section shows mild degree of liver necrosis. Hepatocytes are compact. Hepatic sinusoids appear in normal. The Hepatocytes are well arranged like clusters. There is no inflammation seen, The portal tracts are normal (Fatty change +)

Group - IV

Group - IV Liver tissue section shows hepatocytes with fatty change. Hepatic sinusoids appear in normal. There are areas of lobular inflammation seen along with fatty changes in the perivenular region. There no fibrosis seen (fatty change+++ ,lobular inflammation +)

Group - V Liver (e)

Group - IV Liver tissue section shows that hepatocytes were regenerative and showed no visible changes and prominent nuclei, reduced score of necrosis and there is no inflammation. The portal tract are normal and there is no fibrosis seen.(Fatty change+, No lobular inflammation) Thus, confirming the safety of the extract.

DISCUSSION

Liver injury due to carbon tetrachloride in rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P 450 in endoplasmic reticulum and mitochondria with the formation of $CCl_3O\cdot$, a reactive oxidative free radical, which initiates lipid peroxidation and induces liver damage.

Damage induced in liver by carbon tetrachloride is accompanied by increase in the activity of some serum enzymes. The study of different enzyme activities such as SGOT, SGPT, ALP, TOTAL BILIRUBIN, TOTAL PROTEIN, CHOLESTEROL, TRIGLYCERIDES, HDL, LDL and VLDL have been found to be of great value in the assessment of clinical and experimental liver damage.

The liver is the largest organ in the vertebrate body, and is the major site of xenobiotic metabolism and excretion. Liver injury can be caused by toxic chemicals, drugs, and virus infiltration from ingestion or infection. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems (Karan et al., 1999; Chatterjee, 2000). Carbon tetrachloride (CCl_4) has been widely used in animal models to investigate chemical toxin-induced liver damage. The most remarkable pathological characteristics of CCl_4 -induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl free radicals metabolized by the mixed function cytochrome p450 in the endoplasmic reticulum (Recknagel et al., 1989). Usually, the

extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes (ALT, AST and ALP), thus leads to leakage of large quantities of enzymes into the blood circulation. This was associated by massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Plaa and Charbonneau, 1989).

The aim of the present study was to investigate whether the extract from the fern *Solanum seaforthianum* possesses any preventive as well as curative role against CCl₄ induced hepatic damages. CCl₄ induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and transaminases in circulation. It is well documented that CCl₄ is biotransformed under the action of microsomal cytochrome-P450 of liver to reactive metabolites 31, 32. These free radicals bind covalently to unsaturated lipid membrane, provoking a sharp increase of lipid peroxides followed by pathological changes such as elevated levels of serum marker enzymes like SGOT, SGPT and SALP, depletion of GSH, decreased protein synthesis, triglyceride accumulation, increased lipid peroxidation, destruction of Ca²⁺ homeostasis and finally hepatocyte damage. This suggests that CCl₄ induces liver injury by sharing a common property of free radical mechanism.

Hepatocellular necrosis or membrane damage leads to very high levels of serum SGOT and SGPT released from liver to circulation. Among the two, GPT is a better index of liver injury, since SGPT catalyses the conversion of alanine to pyruvate and glutamate, and released in a similar manner, thus

liver GPT represents 90% of the total enzyme present in the body. The elevated levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane and liver. ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure. It is well known that toxicants like CCl₄ produce sufficient injury to hepatic parenchyma cells to cause elevation in serum bilirubin, and in contrast decrease the level of total plasma protein content.

The activity of serum lipid profile such as total cholesterol, triglycerides, LDL & VLDL were elevated, and this indicated deterioration in hepatic function due to the damage caused by CCl₄ administration. Whereas treatment of *Solanum seaforthianum* extract significantly declined the effect of CCl₄ induced damage and it was evidenced by the decreased level of total cholesterol, triglycerides, LDL & VLDL and increased level of HDL in *Solanum seaforthianum* treated group.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin.

Necrosis or membrane damage releases the enzyme in to circulation and hence it can be measured in serum. A high level of SGOT indicates liver damage, such as that caused by viral hepatitis as well as cardiac infraction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore SGPT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated

levels of serum enzymes are indicative of cellular damage and loss of functional integrity of cell membrane in liver. The ability of the methanolic extract of leaves of *Solanum seaforthianum* prevent the increase in the activities of these enzymes is primary evidence indicative of hepatoprotective activity.

IN VIVO ANTIOXIDANT ENZYMES

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as Catalase, Glutathione peroxidase, Superoxide dismutase and Lipid peroxidase. When the balance between ROS production and antioxidant defence is lost, oxidative stress results through a series of events deregulates the cellular functions leading to various pathological conditions. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues and the highest activity is found in red cells and liver. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and OH. Therefore, the reduction in the activity of catalase may result in a number of deleterious effects due to accumulation of hydrogen peroxide. In the present study, treatment with methanol extract of *Solanum seaforthianum* leaves increased the level of catalase significantly in dose dependent manner and protected the liver from CCl_4 intoxication.

Superoxide dismutase (SOD), a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the methanol extract *Solanum seaforthianum* leaves, significantly increased the SOD activity in CCl₄ intoxicated rats there by diminished CCl₄ induced oxidative damage.

While SOD catalyzes the conversion of superoxide free radical to less toxic hydrogen peroxide, GPx catalyzes the breakdown of hydrogen peroxide into water and oxygen and can also directly detoxify lipid peroxides generated by ROS (Castro and Freeman, 2001). As SOD and GPx are easily inactivated by ROS or lipid peroxides, this may explain a decrease in activities of these two enzymes observed in liver tissue of CCl₄ intoxicated mice in our study. However, our results demonstrated that SOD and GPx were appreciably elevated by MEAI administration, suggesting that it can restore both enzymes and/or activate enzyme activities in CCl₄-damaged liver tissue.

Lipid peroxidation (LPO) has been postulated as being the destructive process in liver injury due to CCl₄ administration. The excessive ROS generated during CCl₄ metabolism rapidly reacts with lipid membranes. This initiates lipid peroxidation chain reaction, which produces lipid peroxy radicals. Enhanced hepatic MDA, a major reactive aldehyde resulting from the peroxidation of poly unsaturated fatty acids in the cell membrane, reflects a casual role of lipid peroxidation in CCl₄-induced liver damage. The increase in

malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with methanol extract of *Solanum seafortianum* leaves exhibited a significant inhibitory role against lipid peroxidation in rats and thereby diminished CCl₄ induced hepatic membrane destruction and hepatic damage. The prevention of lipid peroxidation might, at least in part, be derived from the capability of *Solanum seafortianum* Leaves to scavenge ROS.

The qualitative phytochemical analysis on the methanolic extract of *Solanum seafortianum* shows the presence of flavonoids. (C Pan *Et.al*, 2011).

The dominant constituents of *Solanum seafortianum* the flavonoids and triterpenoids. Flavonoid constituent of plant possess antioxidant and hepatoprotective properties (Wegner T and Fintelmann V, 1999).

7. SUMMARY AND CONCLUSION

The present study was undertaken to determine the hepatoprotective and antioxidant effect of methanolic extract from leaf of *Solanum seaforthianum*

The Pharmacognostical studies made on the powdered leaves of *Solanum seaforthianum* like ash values, extractive value, loss on drying gave valuable information. This will help for correct identification of the plant.

The preliminary phytochemical investigation showed the presence of, alkaloids, glycosides, flavonoids, and steroids, carbohydrate, fatty acids and proteins and phenolic compounds.in methanol extract.

Histopathological studies on isolated liver revealed that methanolic extract of *Solanum seaforthianum* reversed the liver damage caused by CCl₄. The normal pattern of histology of liver was observed.

Phytochemical screening revealed the presence of flavonoids. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect. Example; *Solanum seaforthianum* contain 2-di-C-glycosylflavones of vicenin and lucenin type, anthocyanin-cynidin-3-glucoside,luteolin-7-glycoside and mono-C-glycosyl flavones – vitexin and orientin

Based on the results obtained from the present study, it can be concluded methanolic extract of *Solanum seaforthianum* is found to be more potent and effective hepatoprotective and antioxidant activity.

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